

**AMELIORATION OF TRANSPORTATION STRESS BY
ANTI-STRESS SUPPLEMENTATION AND LAIRAGE
ENVIRONMENT FOR BETTER MEAT QUALITY AND
WELFARE OF BROILER CHICKEN DURING SUMMER**

Thesis

**Submitted to the
DEEMED UNIVERSITY
Indian Veterinary Research Institute
Izatnagar - 243 122 (U.P.), India**

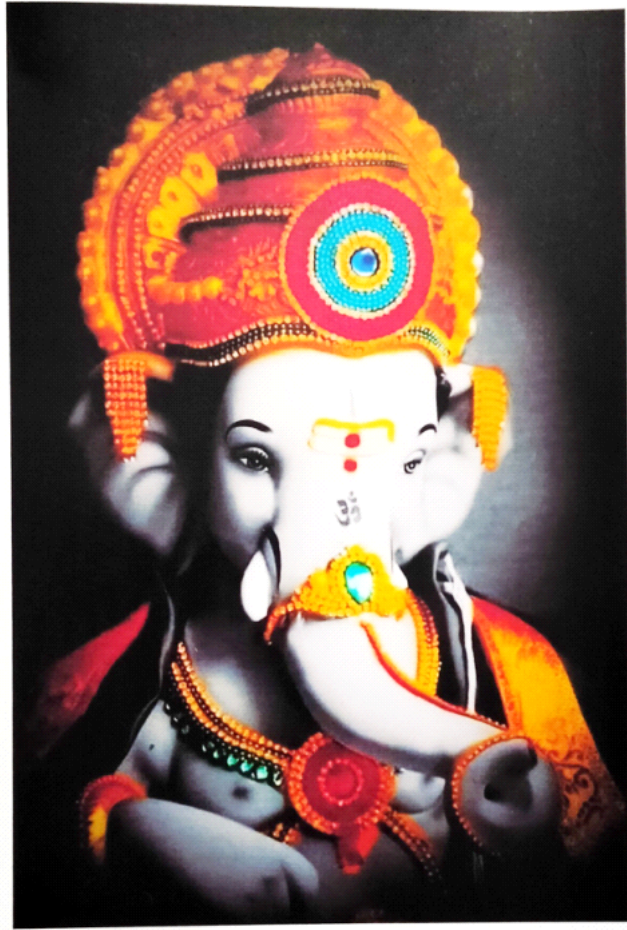


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Roll No. 6240**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE DEGREE OF**

**Master of Veterinary Science
(Poultry Science)**

2023



Dedicated to...

*My Beloved Family
Respected Guide
and
Dear Friends*





भा.कृ.अनु.प.-केन्द्रीय पक्षी अनुसंधान संस्थान
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Certificate

Certified that the research work embodied in this thesis entitled “Amelioration of transportation stress by anti-stress supplementation and lairage environment for better meat quality and welfare of broiler chicken during summer” submitted by Dr. Prashant Patel, Roll No. M-6240, for the award of Master of Veterinary Science degree in Poultry Science at ICAR-Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.

It is further certified that Dr. Prashant Patel, Roll No. M-6240 has worked for more than 21 months in this Institute and has put in more than 150 days attendance under me from the date of registration for the degree of Master of Veterinary Science of the Deemed University, ICAR-Indian Veterinary Research Institute as required under the relevant ordinance.

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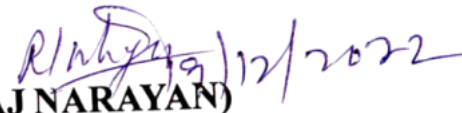
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We the undersigned members of Advisory Committee of **Dr. Prashant Patel, Roll No. M-6240**, a candidate for the degree of **Master of Veterinary Science with the major discipline in Poultry Science**, agree that the thesis entitled "**Amelioration of transportation stress by anti-stress supplementation and lairage environment for better meat quality and welfare of broiler chicken during summer**" may be submitted in partial fulfillment of the requirement for the degree.

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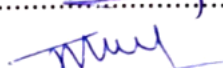
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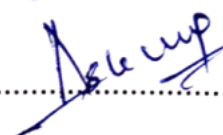
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Prashant

Date: 9 - 1 - 2023

(Prashant Patel)

Place: ICAR-IVRI, Izatnagar

ABBREVIATIONS

%	: Percentage
°C	: Degree centigrade
µl	: Microliter
ALT	: Alanine aminotransferase
AST	: Aspartate aminotransferase
BAHS	: Basic Animal Husbandry Statistics
DADF	: Department of Animal Husbandry Dairying and Fisheries
DOA	: Dead On Arrival
FAO	: Food and Agricultural Organization
g/L	: Gram Per Liter
GDP	: Gross Domestic Product
H/L	: Heterophil/Lymphocyte
hr	: hour
mg	: Milligram
ml	: Millilitre
NAP	: National Action Plan
NGO	: Non-Governmental Organization
pH	: power of Hydrogen
pHu	: Ultimate pH
ppm	: parts per million
RA	: RunAway
rpm	: Revolutions per minute
SDS	: Sudden Death Syndrome
SFV	: Shear Force Value
T	: Treatment
TI	: Tonic Immobility
UP	: Uttar Pradesh
WB	: Warner Bratzler

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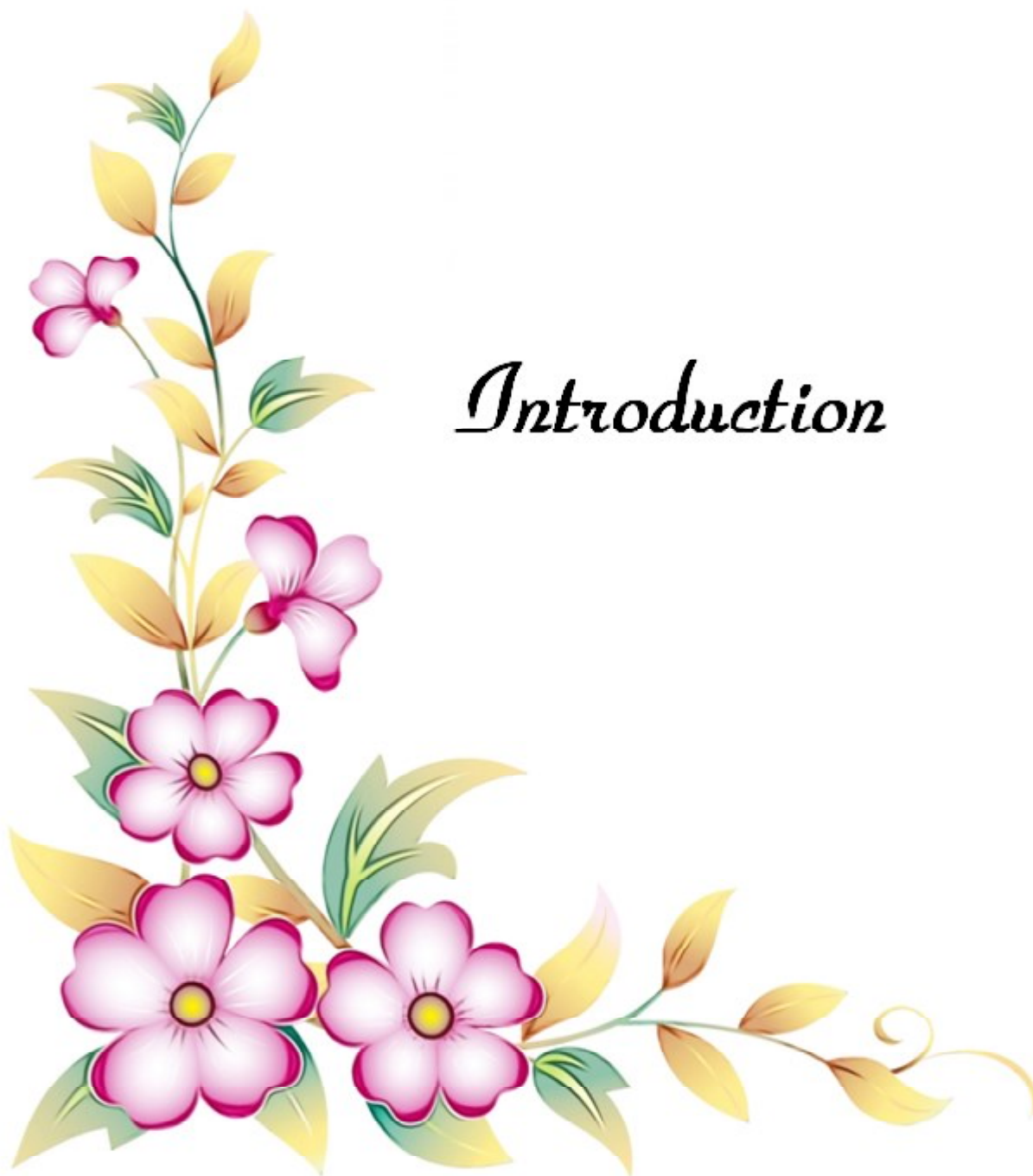
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Introduction



The poultry industry is one of the well-organized sectors, ranging from backyard poultry farming to commercial poultry farming. Livestock farming has been conducted all over the world since ancient times to provide for the nutritional needs of the human population. The demand for animal products is growing every day, thanks to the rising population, urbanization, most importantly, people's growing interest in healthy diet plans especially animal origin protein intake. In this age of globalization, the chicken industry has emerged as a forerunner in the food industry, and its growth rates are skyrocketing. Factors such as cheaper inputs and greater protein outputs, both qualitatively and quantitatively, could be contributing to the poultry sector's phenomenal rise. The total egg production and poultry meat production of our country are 122.05 billion and 4.47 million tonnes respectively (BAHS, 2022). India rank 3rd in terms of total egg production and 8th in terms of total meat production (BAHS, 2022). In India, poultry accounts for more than half of the country's total meat production. The growth of poultry egg production has increased by 6.70% while poultry meat production has decreased by 2.95% over the previous year (BAHS, 2022). This growth in egg production is far higher than the 2.9% growth rate of agricultural crops (Economic Survey, 2019-20). The availability of protein from chicken meat is anticipated to rise by 17.8% by 2030, accounting for 41% of all meat-based protein sources worldwide (FAO, 2021). By 2021-2030, the consumption of poultry meat is anticipated to increase to 152 million tonnes, making up 52% of the total increase in meat consumption (FAO, 2021). According to the AIPBA, the poultry sector contributes USD 17.31 billion to India's total gross value and employs 50 million people directly or indirectly. Broilers account for 65.3% of the poultry business, with 400 million chicks produced monthly

(ICRA, 2020). Simultaneously, the layer business accounts for 34.7% of the chicken industry, producing around 8,400 million eggs each month (ICRA, 2020). More than 1 million farmers work in the poultry industry, with more than 85% of them owning less than 2 hectares of land. The poultry industry's rise has been hailed as a major milestone in the country, with 851.8 million birds produced annually. According to the 20th livestock census conducted in 2019, the population of poultry is 851.81 million, increasing 16.81 percent from the previous census. The commercial population is estimated to be 534.74 million, with a 4.5 percent growth rate. The Indian broiler sector is quickly expanding as a result of rising demand. Feeding, breeding, housing management, and health management have all benefited from the combination of technology and traditional ways. Corporate investments in the poultry industry have also accelerated the sector's growth. This sector has also benefited from technological training for rural residents.

Among all the livestock sectors prevalent in India, poultry is considered as the most organized, highly mechanized, and industrialized sector. There is a large diversification in it ranging from conventional backyard poultry to organized poultry rearing. The GDP contribution is higher from broilers as compared to the layer industry (DADE, NAP for egg and poultry-2022). The main reason responsible for this is the ease in the rearing of broilers than layers. It also requires low investment costs, short market age, and poultry companies have also integrated poultry farms with them. Due to the intense genetic selection, broilers are the fastest-growing species among farm animals. Consumer prefers poultry meat nowadays due to no religious taboos in it, changing food habits, and an increase in per capita income. This has led to the growth of the broiler sector. The market weight of broilers is around 2kg which is achieved after rearing them for 35 to 42 days. The nutritive value of poultry meat is significant and it is a good source of proteins, vitamins, and minerals. Omega-3 fatty acids and vitamin B12 is also present in adequate amount in poultry meat with a low amount of trans fat making it a healthy option. Extra dietary nutrients can be added to meat if needed. Live poultry held 90-95% of the total share. They are purchased for the slaughter and further processing of poultry products by the integrators as well as by the retailers and wholesalers for the sale in the market. Due to its low cost, chicken meat is accessible to middle-class families in India which are making this

sector prosper and enormous growth seen. As the population of India is very large, even a slight increase in consumption booms the sale of the birds.

In spite of great success poultry in India have many challenges to face like welfare, feed cost, stress leading to loss of production, AMR, welfare etc. Stress is nothing but detrimental effects of factors like disease, environmental fluctuations, changing seasons, poor feed quality, transportation, and many more which deteriorate the health and performance of poultry. Other factors which contribute to stress in poultry are vaccination, any disease from which they suffer, noise, speed, the micro-thermal temperature of the vehicle, mixing of unknown groups, stocking density and feed, and water withdrawal during transportation and sometimes the vehicle itself becomes inappropriate for transportation. These factors initiate from slight discomfort in birds and ultimately to death if not taken care of. To compensate for stress, their bodies secrete chemicals and hormones like adrenaline and cortisol. Birds give their best performance when they are in their comfort zone of temperature, any increase or decrease will lead to decreased production and health status. Firstly, the bird tries to adapt to the change by initiating physiological changes in its body. But when conditions go beyond, they suffer from stress. We should give our best efforts to avoid stress for them by providing proper care, environmental, and managerial practices.

Even though there has been significant growth in the poultry sector, the welfare of the birds has not been acknowledged due to high stocking density in farms, poor housing, and transport conditions (Bessei, 2006). Many welfare related issues remain unaddressed even today in the broilers due to the genetic selection, scientific feeding, and management of broilers to grow at a faster rate for intensive production (Bessei, 2006). A faster growth rate due to intensive selection in broilers leads to the diseases like Sudden Death Syndrome (SDS) and other metabolic diseases like ascites. Leg weakness is also reported. Other factors such as the stocking density of broilers, quality of litter, and ammonia concentration in the air also affect the welfare along with the high growth rates. There is a requirement for faster growth rates in poultry to gain economic benefits, and because of that compromised welfare is commonly accepted especially in developing countries. Broilers also suffer injuries of high levels during loading and transport. This creates an increased risk of mortality in birds. Consumers have

now started raising issues about the welfare of birds and food quality which has led to the need for welfare assessment for commercially raised poultry. Here, the concept of animal welfare comes into action. It is now a need of an hour to know what animal welfare really means when we talk about the welfare of animals.

The Farm Animal Welfare Council, 1993 guides the welfare by five freedoms: Freedom from thirst and hunger, Freedom from discomfort, Freedom from injury, pain, or disease, Freedom to express normal behaviour, and freedom from distress and fear. All these freedoms are applicable to poultry too. Most of the commercial broilers are reared in those industrial farms which have less floor space and are transported in much poorer conditions than a live bird to market (Kolluri, 2021). Thus during transport and rearing, the broiler's welfare is compromised to a great extent. Metabolic disorders, leg problems, lameness, breast burn, and transport stress (Jong and Guemene, 2011) comprise the main issues related to welfare. The most common mode of transportation of birds is through roads all over the year. Stress while transportation deteriorates carcass quality, also economic development and animal welfare get hampered (Schtte, 1994). Overall body homeostasis is disturbed because of change in physiological, biochemical, immunological, and hormonal function (Zhang *et al.*, 2009). If bird is already suffering from transportation stress and above that if managemental practices are also not proper like feed withdrawal and improper handling, etc. affects the expression of hepatic gene which is correlated with cellular control, immune function, and nutrient metabolism (Sherlock *et al.*, 2012). Endogenous contamination by microbes increases in broilers while transportation which directly affects the meat which we are consuming (Mengert *et al.*, 1996). That's why it is now a major concern for reducing transport stress. Wang *et al* in 2016 suggested that using water-misting spray along with forced ventilation treatment has proved a better way to conserve meat quality of broilers without the use of any chemical during summers as it has detrimental effects in summer season transportation.

The welfare of broilers is compromised to a great extent during transportation in those conditions which are prevalent in India because in India, birds are mostly transported during the daytime without any shade in mostly bad road conditions. Transport stress is more when birds are fetched during the hotter part of the day along with the withdrawal of feed/water and

other disturbances like noises and vibrations from the vehicle. This alters the bird's normal physiology, resulting in increased weight loss and mortalities during or after transportation. Physiologically, transport affects the muscle pH to a greater extent and results in the depletion of muscle glycogen reserves. The bird's physiology is affected by transport stress and causes fearful responses which in turn affects the meat quality and causes weight loss (Mitchell and Kettlewell, 2009). In the conditions prevalent in India, different vehicles like bicycles, motorcycles, and trucks are used to transport broilers. The transport stress is significantly affected by the duration of transport between the farms to slaughterhouses. In India, two wheeler vehicles without crates/container are also used to transport birds at smaller level. Stress caused by transport of broilers can be ascertained based upon measurements of tonic immobility (TI), H/L ratio, HSP-70 and plasma corticosterone concentrations. On the other hand, this stressful condition leads to excessive growth of pathogens (Bello *et al.*, 2018) and affects the normal intestinal microbiota.

Nutritional modifications have been tried as one technique for minimizing stress in chickens. In stressed birds, providing several feed additives such as vitamin C, E, betaine, aspirin, and electrolytes, etc. has been successfully tried to minimize the effect of stress (Asad *et al.*, 2019). There are so many preventative stressors available that can be tried in stress conditions like heat stress, especially in tropical countries like India. These proven antistressors can also be used in transportation-induced stress to ameliorate the side effect of transportation stress. To check whether the bird is in good welfare condition or not, broiler welfare indicators during transport have to be analysed, and also identified the effects of antistressors to reduce the effect of stress. But for the Indian scenario, the research works on broiler transport and stress amelioration by different antistressors are scanty. Also, lairage environment before slaughter acts as a recovery period after transportation stress and prevents the bird from more stress. Not much research work has been carried out on pre-slaughter resting environment. So, it is necessary to study the resting period before slaughter to assess their effects on the carcass.

Blood profile analysis, stress index, plumage condition, foot pad index, weight loss, carcass quality, and microbial load (Ulupi *et al.*, 2018) are used to identify broiler welfare

indicators during transport. Broilers are prone to handling and transport stress during transport which ultimately compromises the chicken's welfare. Therefore, in order to identify the welfare status of the bird the above said welfare indicators are adopted. From the interpretations of this study, positive recommendations can be made for better broiler welfare. Keeping in view the above facts, the present research work is put forward to ameliorate broilers transport stress by evaluating lairage environment & antistressor supplementation effect:

Objectives

- 1. To evaluate the antistressor package supplementation and lairage environment on production, welfare and HSP-70 gene expression in transport stressed broiler chickens.**
- 2. To evaluate the effect of antistressor package supplementation and lairage environment on meat quality and physio-biochemical attributes.**



*Review
of
Literature*



2.1. Concepts of Poultry Welfare

Poultry meat and eggs are the most widely consumed animal source foods worldwide, owing to a vast range of cultures, traditions, and religions, making them essential for food security and nutrition. Within the livestock industry, poultry is the most resource efficient sub-sector in terms of using natural resources and meeting world protein demand. During the previous four decades, the global poultry industry has experienced tremendous growth and development. Genetics, nutrition, and husbandry advancements have resulted in a significant increase in productivity.

As a result, the Indian poultry business has become one of the most dynamic and fastest-growing segments in animal husbandry, with fair growth rates in broiler meat and egg production. Animal welfare, according to the World Organization for Animal Health (OIE), is defined as an animal's ability to cope with the conditions in which it lives. It encompasses both physical and mental well-being and involves a variety of factors such as physical comfort, the absence of hunger and disease, the ability to engage in motivating activities, and so on. The Brambell Committee in the United Kingdom published a study in 1965 proposing that farm animals be allowed to turn around, lie down, rise up, stretch, and groom without limitation (Brambell, 1965).

According to the five freedom concept, animals should be “freedom from hunger and thirst, freedom from discomfort, freedom from pain and disease, freedom from fear and distress and freedom to express normal behaviour”. Any deviation from this concept results in welfare

issues in birds. All of the major chicken species and breeds used in the main intensive production systems are moved at least twice during their lives, across distances ranging from a few kilometres to several hours. The majority of journeys are made by road, such as from the hatchery to the production site or from the farm to the processing plant. All routes of transportation entail the loading of birds or chicks into transport containers, which are then put onto vehicles to be transported to intermediate or final destinations.

2.2. Poultry Transport

Transportation is an important part of the commercial poultry sector. It creates a variety of stress levels in birds, ranging from minor pain to death. Numerous factors such as bird's health before transport, handling, loading, unloading, vehicle design, loading density, environmental condition during transportation, vehicle microclimate, transit duration, lairage duration, and length of feed withdrawal affected the chicken's welfare.

Transport micro-environment, acceleration, vibration, motion, fasting, withdrawal of water, social disruption, and noise are all possible stressors for birds in transportation (Abeyesinghe *et al.*, 2001). Each of these stressors and their combination may cause stress to the birds, but it is well acknowledged that thermal difficulties, particularly heat stress, pose the greatest threat to bird well-being and productivity (Mitchell, 2006). The application of thermal stress to birds in transit causes medium to severe thermal stress, resulting in reduced welfare (Mitchell *et al.*, 2001), increased mortality due to heat or cold stress (Hunter *et al.*, 1999), and induced pathology, such as muscle damage and associated changes in meat quality (Gregory 1998, Mitchell 1999). However, in 2014 Zhang *et al.*, and in 2016 Wang *et al.*, reported that stress can be mitigated by dietary supplements like providing nutrients like creatine monohydrate or can be vitamin C and chromium (Peraí *et al.*, 2014). And birds become susceptible to disease by impairment of immune function (Hansson *et al.*, 2005). Studies suggested by Bayliss and Hinton in 1990 said that 40% of birds that are dead on arrival are because of transportation stress. It not only affects meat quality but productivity also goes down (Fellenberg and Speisky, 2006, Franco-Jimenez and Beck, 2007, Rozenboim *et al.* 2007; Ajakaiye *et al.*, 2010). It is clear that the impacts of transportation on birds will be determined by climatic circumstances,

which will influence the internal vehicle's thermal micro-environment. The duration of the travel has an impact on the effects of any adverse "on-board" thermal conditions. In all nations where meat birds are produced, mortality has long been a concern in relation to poultry transportation (Bayliss and Hinton, 1990) and continues to be a recurrent issue. According to Warriss *et al.* (2005), the highest daily ambient temperature has a significant link with broiler mortality during transport (DOAs) or in lairage. According to Ritz *et al.* (2005), broilers are at a higher risk of pre-slaughter mortality during loading and unloading at the abattoir during warmer days.

Nijdam *et al.* (2004) identified that DOA can be easily lowered by lowering the stocking density of birds, with each additional bird in box/ container increasing the number of DOA by 1.1%. The length of the journey has been identified as a significant determinant in broiler DOA (Warriss *et al.*, 1990). Vecerek *et al.* (2006) found that short journeys (up to 50 km in duration) are connected with relatively low mortalities (0.15 percent), but journeys of 300 km or more are related with 0.86 percent. Whiting *et al.* (2007) reported travel time has a significant impact on DOA rates and 10.5% of the variation was observed on the DOA rate when the time of slaughter, shrink, and transport time was combined. According to Vosmerova *et al.* (2010), shorter transit durations may be more painful for the birds because they do not have enough time to recover from the stress caused during handling and crating, which has been demonstrated to be the most stressful of all sources of stress. Transportation time has been shown to have a considerable impact on plasma glucose levels and breast muscle glycogen levels (Vosmerova *et al.*, 2010, Zhang *et al.*, 2009). Long-term transit resulted in a decrease in breast muscle glycogen content (Savenije *et al.*, 2002, Zhang *et al.*, 2009).

Sandercock *et al.* (2006) identified that fast-growing broilers strains have a higher incidence of spontaneous or idiopathic myopathy, as well as a higher risk of stress-induced myopathy, and these are caused by abnormalities in intracellular calcium homeostasis resulting changes in sarcolemmal integrity and may be caused by myofibrillar hypertrophy and inadequate vascular supply (MacRae *et al.*, 2007). These myopathies affect both meat quality and the welfare of broilers (Mitchell, 1999). Sandercock *et al.* (2006) identified that rapidly growing lines may have a lower thermoregulatory capability than their ancestors, making them more sensitive to heat stress in transit and problems like muscle injury, acid-base

disturbances, and poor meat quality. Genetic selection for better growth rate and feed conversion efficiency has been linked to alterations in mitochondrial function and reactive oxygen species generation (Bottje *et al.* 2006). Mujahid *et al.* (2005) found that acute heat stress increases superoxide free radical generation in chicken skeletal muscle by change in mitochondrial function and a decrease in “uncoupling protein content”. This could be the reason for broiler muscle damage caused by transportation stress and heat stress and lead to changes in muscle and meat quality. Environmental variables have been shown to alter the color and pH of thigh meat more than breast meat in broilers (Watts *et al.* 2011). The stress caused by capturing and loading the birds before transportation is greater than the stress caused by the transportation itself (Voslarova *et al.*, 2011). Voslarova *et al.* (2011) found that corticosterone levels increased for 7 minutes after birds were placed in cages, then began to fall, but not to levels seen in non-caged birds even after 2 hours. Chloupek and colleagues (2008), concluded that plasma cortisol levels increased three-fold following a four-hour caging period

2.3. Effect of transport on different parameters

According to Shuzhen *et al.* (2021) decreased levels of T3 and T4 were observed after 2 hr of transport. This decrease is due to the release of corticosterone in chickens which leads to reduced synthesis of T4 and T3 and also inhibits the conversion of T4 to T3 by suppression of 5 deiodinase enzyme activity. The 2 h transit stress resulted in a drop in serum insulin levels by stimulation of the sympathetic nervous-adrenal medulla and limited insulin production, resulting in glucose metabolic alterations. Yue *et al.* (2010) found that as the travel time increased, the drip loss from the breast and thigh muscle increased significantly. Shuzhen *et al.* (2021) found that the initial glycogen reserves of muscle determined the ultimate pH of meat which is an important factor for the conversion of muscle to meat.

Vecerek *et al.* (2006) found that distance travelled results in the variation of mortality of broilers. Long-distance transport increases the death rates in broilers (DOA). Ehinger, (1977) identified that increased transit length and crates space resulted in a higher amount of bruising. Weeks and Nicol (2000) concluded that pre-slaughter rest allows animals to recover from stress. Sams, (1999) reported that stress hastens the onset of rigor mortis, PM glycolysis,

and metabolic alterations in muscle resulting in changes in meat quality. Bianchi *et al.* (2006) reported that a decrease in pH and water holding capacity of meat and also an increase in drip loss, cooking loss were observed in acute stress. Debut *et al.* (2003) observed that depletion of muscle glycogen reserves was observed during stress which ultimately affects the meat quality. According to Pan *et al.* (2018) transportation of broilers for 3 hrs causes a marked reduction in live BW. Warriss *et al.* (1990) identified that loss of BW after transit was due to dehydration and tissue mobilization for energy. Shini *et al.* (2009) reported that blood glucose level was elevated in the long-distance travelled group due to activation of the hypothalamic pituitary adrenal axis.

2.4. Antistressor

Antistressor is a product that helps chickens cope with the negative effects of numerous stresses. Till now so many antistressors have been used in the broiler industry to reduce the effect of stress. Vitamins, minerals, and herbal compounds have the potential to ameliorate the effects of stress in chickens. Vitamins mainly A, D, E, and C have been shown to have a great impact on immune system function in a variety of ways. Vitamin E and C both are known for their antioxidant and anti-inflammatory property during stress conditions. Pandurang *et al.* (2011) reported that feeding of anti-stressor during stress decreases the negative impact of the stressor. Whitehead and Keller (2003) reported that supplementation of feed additives like vitamin C, betaine, and electrolytes showed positive response against stress. Attia *et al.* (2009) found significant ($P < 0.05$) body weight gain, FCR, and feed intake in broilers when supplemented with AA and Betaine.

2.4.1. Vitamin C

L-ascorbic acid is a white crystalline substance that is produced endogenously from the chicken's kidney but endogenous ascorbic acid is insufficient to meet the bird's needs during stress. It's a 6-carbon lactone produced by poultry birds from glucose. In birds, vitamin C improves immunity, feed intake, weight gain, oxidative stress, rectal and body temperature, fertility and semen quality, carcass weight, and mortality caused by stress. Vitamin C is necessary for the manufacture of corticosterone, a hormone that increases energy supply during times of

stress. Nockels (1984) reported that physiological stressors increase the need for vitamin C in birds. For proper metabolism of minerals, AAs, and synthesis and secretion of hormones that act as antistressors vitamin c is required. Vitamin C supplementation in the diet has been shown to reduce the expression of inflammatory cytokines against oxidative stress (El-Senousey *et al.*, 2018).

2.4.1.1 Effect of Vitamin C supplementation on different parameters during stress

Ascorbic acid therapy lowers the rectal temperature, enhances feed intake, and also boosts body weight gain (Bolukbasi *et al.*, 2006). Kadim *et al.* (2008) reported that feeding of ascorbic acid at 200-300 ppm increases feed conversion, feed intake, and body weight gain by 5%, 8%, and 11% respectively. The addition of vitamin C in drinking water (@ 20 mg/kg/bird/day) showed an increase in body weight by 10.9% in chickens during acute heat stress (Vathana *et al.*, 2002). Richard (1998) reported that Vitamin C supplementation reduces the level of plasma corticosterone in birds. Supplementation of vitamin C helps in the synthesis of phagocytes and neutrophils during stress, which are crucial components of a chicken's immune system (Vathana *et al.*, 2002). Tuekam *et al.* (1994) identified a positive correlation between ascorbic acid supplementation and antibody titer. According to Quarles and Adrian (1989) supplementation of ascorbic acid (976 ppm/128 gallons) in drinking water increases the carcass yield. According to Peebles and Brake (1985), a 14.6% reduction in mortality rate was observed during heat stress when birds were supplemented with 1000 ppm of Vitamin C. Attia *et al.* (2009) found supplementation of AA and different doses of Betaine caused significant improvement in dressing percentage.

Minka and Ayo (2008) concluded that the H/L ratio is the most dependable measure for predicting the long-term effects of several stressors in poultry. Minka and ayo conducted research on transportation stress and found that the H/L ratio was significantly higher in the group transported without any antistressor and recorded a high H/L ratio in pullets immediately after 3 hr and 3-day post transportation. This increased H/L ratio showed stress in birds. They also found that 20% mortality was recorded in the control group as compared to the 2.5 and 0% Vitamin E and C supplemented group. Sahin and Kucuk (2001) found the increased performance of birds after supplementation of ascorbic acid under stress condition.

Metheringham and Hubrecht (1996) found that about 35% of injuries have been responsible for the dead-on-arrival of broilers in slaughtering plants. Minka and Ayo (2008) reported that the reduction of live weight after transportation was due to longer food and water deprivation, and muscle exhaustion. They found that groups supplemented with vitamin C showed minor weight loss, this indicated that Vitamin C reduces live-weight loss. According to Vathana *et al.* (2002) effect of heat stress in broilers was significantly reduced. Attia *et al.* (2009) found the highest mortality percentage in the non-supplemented group as compared to Betaine and AA supplemented group. Kumar *et al.* (2017) demonstrated that the supplementation of ascorbic acid reduced mRNA expression of HSP70 in the liver of ducks exposed to stress.

2.4.2. Betaine

Betaine, commonly known as trimethylglycine, is a quaternary ammonium molecule with a zwitterionic structure. Craig (2004) found osmolytic role of betaine. Betaine is a multi-nutritional agent that helps chickens to cope with stress condition. Osmolytes are osmotically active solutes that aid in cell volume maintenance. It can accumulate in cells without interfering with the normal function (Yancey *et al.*, 1982). Another role of betaine is as a methyl donor in the production of methionine from homocysteine. S-adenosylmethionine (SAME) is formed from methionine and is used as a methyl donor in most methylation reactions in the body (Craig, 2004).

2.4.2.1. Effect of Betaine supplementation on different parameters during stress

Mcdevitt *et al.* (2000) reported that hens supplemented with betaine showed improved egg production and eggshell quality. Betaine supplementation improves meat quality and lipid metabolism. Betaine also helps in intestinal function and growth. Ayo *et al.* (2014) reported that shorter tonic immobility was observed in the betaine supplemented group as compared to the control group exposed to stress. According to Amer *et al.* (2018), TI has been identified as an index of fear, and also the duration of TI determines the stress level. Ramnath *et al.* (2008) found that heat stress in chickens lowered antioxidant activities (SOD and GPx). Alirezai *et al.* (2012) concluded that supplementation of betaine increases SOD and GPx

activities in the meat of the broiler as a result of oxidative stress.

Ezzat *et al.* (2011) concluded that a combination of Betaine (1 g/kg diet), vitamin C (200 mg/kg diet), and folic acid (1 mg/kg diet) improved reproduction and performance in stress. Park *et al.* (2006) reported that supplementation of betaine at 600 ppm improved the performance of laying birds and leads to an increase in production. Sayed and Downing (2011) identified that supplementation of betaine (500 mg/L) in drinking water enhanced the body weight of broiler chicks during stress. Ducks when fed betaine supplemented diet showed improvements in growth, FCR, and immune response (Wang *et al.*, 2004). Kumar *et al.* (2017) demonstrated that the supplementation of betaine and ascorbic acid reduced mRNA expression of HSP70 in the liver of ducks exposed to heat stress and corticosterone concentration in blood.

Jahanian and Rahmani (2008) reported that betaine inclusion in the diet of broiler birds improved the carcass yield and breast % with decreased abdominal fat. Akhavan-salamat and Ghasemi (2016) identified that betaine is much more effective against stress in heat-stressed broilers. Shakeri *et al.* (2018) found that betaine supplementation improved the performance of chickens. Al-Abdullatif *et al.* (2021) Supplementation of betaine in drinking water (1 ml/L, 2 ml/L) 72 hr before transportation showed a beneficial effect against stress. Al-Abdullatif *et al.* (2021) found that betaine supplementation has not had any effect on WHC, cooking loss, meat quality, and pre-slaughter transit. Park and Kim (2019) reported that no effect of betaine supplementation on broiler performance. According to Chen *et al.* (2018), betaine feeding improved the FCR of broilers. Attia *et al.* (2009) found supplementation of AA and different doses of Betaine reduced the negative effect of heat stress and significant ($P < 0.05$) improvement in rectal temperature. Esteve and Mack (2000) found supplementation of different doses of Betaine caused significant improvement in eviscerated percentage.

2.4.3. Glucose and its effect on different parameters during stress

Most important nutrient for cellular energy. Karacay *et al.* (2008) found the significant effect of glucose supplementation with drinking water during feed withdrawal on live weight losses, carcass yield, and meat colour. According to Nissen and Young (2006), the amount of

energy stored in the muscle at the moment of slaughter and the rate at which it depletes post-mortem affects meat quality. According to Bhanja *et al.* (2004), in-ovo feeding enhanced the weight of hatchability and the pace of body growth of chicks. However, in another investigation, it was discovered that injecting glucose into albumin reduced the percentage hatchability of chicks (Pedroso *et al.*, 2006; Bhanja *et al.*, 2008). When glucose was mixed with water and given to birds under heat stress, the thermoregulatory system in birds performed more efficiently, but when the thermoregulatory ability of birds was insufficient, the mortality of birds was enhanced, according to Zhou *et al.* (1998).

Iwasaki *et al.* (1997) identified that mortality in birds consuming glucose water was less when compared with birds that consumed water alone. According to Zhou *et al.* (1998), chickens consuming glucose water have more efficient thermoregulatory functions than those consuming water alone under stress conditions. Talebi *et al.* (2011) stated that the addition of Vitamin C and glucose in drinking water decreases the effect of high temperature on the grower and finisher stage. Thaxton *et al.* (1974) identified that oral administration of glucose water in newly born chicks increases body temperature. Hernawan *et al.* (2012) concluded that the oxidation of glucose leads to the yield of glycogen, amino acids, fatty acids, vitamin C, and a variety of other metabolites, and also produces energy.

2.4.4. Aspirin

The primary constituent of aspirin is acetylsalicylic acid (ASA), which acts as an antipyretic agent (Weissmann, 1991). The effectiveness of aspirin depends upon its ability to reduce prostaglandin. This effectiveness is done by the irreversible blocking of cyclooxygenase (prostaglandin synthase) which helps in the conversion of arachidonic acid to endoperoxide compounds, at appropriate doses the drug decreases the formation of both prostaglandins and thromboxane A₂ but not the leukotrienes. Aspirin blocks uterine contractility. Few researchers had studied the effect of aspirin on growth and egg production.

2.4.4.1 Effect of Aspirin supplementation on different parameters during stress-

According to Glick (1963) supplementation of aspirin at 0.3% in the diet showed improved growth. Hens supplemented with 0.05% aspirin improves egg production, feed efficiency, and shell % (Thomas *et al.*, 1966). Dietary supplementation of aspirin has modulating

effect on thyroid activity and concentration of thyroid hormone. According to May and McNaughton (1980), Chickens fed with aspirin had considerably lower serum T3 concentrations than controls while, serum T4 concentrations were not altered. Interleukin (IL-1) and tumour necrosis factor (TNF- α) are two cytokines that are activated by ASA (Yoo *et al.*, 2001).

Aspirin has been shown to stimulate the production of ferritin, an antioxidant defence protein. Ferritin sequesters iron that would otherwise catalyse detrimental oxidative processes, in addition to functioning as an iron reserve for the preservation of important cellular functions. Ferritin's cytoprotective antioxidant action was mainly unknown until it was discovered that cells overexpressing this protein are more resistant to oxidative harm (Oberle *et al.*, 1996). According to (Shi *et al.*, 1999), aspirin is a powerful radical scavenger that outperforms some well-known antioxidants such as ascorbate, glutathione, and cysteine. Aspirin, on the other hand, seems to protect against silica-induced lipid peroxidation and DNA strand breaking. Aspirin also stopped silica, lipopolysaccharide, and the transition metal Fe from activating nuclear transcription factor-b (II). Some of its physiological and pharmacological activities may be explained by its antioxidant capabilities (Shi *et al.*, 1999). The addition of ASA helps in the reduction of the H/L ratio by reducing the corticosterone level and consequently reduces the heat stress during summer (Abou El-Soud *et al.*, 2006). According to Mohammed (2010) the H/L ratios of Birds supplied with 1 and 1.5 g/L ASA in water were shown significantly ($p < 0.05$) less than the control group.



*Materials
and
Methods*



The proposed research work entitled “**Amelioration of transportation stress by anti-stress supplementation and lairage environment for better meat quality and welfare of broiler chicken during summer**” was conducted in the division of Poultry Housing and Management, ICAR-Central Avian Research Institute, Izatnagar, Bareilly-243122 (28.3670°N, 79.4304°E), Uttar Pradesh. Present experiments were conducted during July-August considered this period as hot-humid summer. The study was carried out as per the guidelines and approval of the Institutional Animal Ethics Committee (IAEC). In this chapter, a brief description of experimental design, bird housing, feeding, management, and techniques applied for assessing the production, welfare, carcass quality, serum biochemical, expression of HSP-70 gene were explained. Also, statistical methods used for the analysis of data are being described.

For the evaluation of the effect of **antistressor package supplementation and lairage environment on transport stressed broiler birds**, this experiment was conducted on 42 day old Cobb broiler chickens procured from the Experimental Broiler farm of ICAR-CARI.

The detailed experimental procedures for the trial were as follows.

3.1.1 Experimental Housing and Management:

Broiler chicks were reared in a deep litter housing system in the Experimental Broiler farm at Central Avian Research Institute, Izatnagar, Bareilly (UP) for a period of 42 days. All management activities like brooding, feeding, watering, etc. were practiced under the same

housing system. Three- dietary rations were offered to the chicks i.e. starter, grower, and finisher diets. The broilers were supplied with *ad libitum* feed and water. On the 42nd day broiler, birds were randomly selected, divided into six treatment groups, and transported for 8 hours. Before transportation birds were kept off feed for 12hr. The last three treatment groups were provided with an antistressor agent via drinking water 3 days prior to transport to assess the effect of the agent on transport stress in broiler chicken.

3.1.2 Experimental Design

Treatment Arrangement: Two Hundred and forty (n=240) Cobb broiler chickens of 42-day old were divided into six treatment groups with each group consisting of forty birds (Four replicates per treatment with 10 birds/replicate). Treatment group T₁ was kept as control and transported for the 8 hour duration and the birds in the treatment groups T₂ and T₃ were transported for 8 hours duration and after transport they were given 1 hr lairage rest under normal lairage temperature and cooler lairage temperature (20-25°C). Three days prior to transport last three treatment groups (T₄, T₅ & T₆) were supplemented (@4.2 g/L, twice a day) with an antistressor and on 43rd day transported for duration of 8hr. After transport treatment groups T₅ and T₆ were given a 1 hr lairage rest under a normal lairage temperature and cooler lairage temperature (20-25°C) respectively. The transportation duration of 8hr and 1 hr lairage rest was decided based on the previous research work which indicates the broiler started showing significant signs of stress after 4hr of transport (CARI annual report 2020). The birds were divided into four replicates per treatment and ten birds in each replicate. Factorial Design (Two-way ANOVA) was followed. On the night of the 42nd day, all the two hundred and forty broiler chickens were divided into six treatment groups as mentioned above and they were not provided with feed and water during transport. The birds were painted with different colours as per treatment for identification. Also, the remaining space in the vehicle was filled up with extra birds so to create an authentic field-like scenario for stress.

After transport, response criteria were analysed from the samples collected from the birds from different treatment groups. The following parameters were analysed as response criteria following transport.



Fig. 3.1: Housing condition of broilers



Fig. 3.2: Antistressor package supplementation via drinking water



Fig. 3.3: Loading of broilers before transport



Fig. 3.4: Unloading of broilers after transport



Fig. 3.5: Normal lairage environment before slaughter



Fig. 3.6: Cooler lairage environment before slaughter

Transport Conditions: The broiler chickens were transported on the 43rd day (5.00 am to 1.00 pm, IST) in a medium-sized chicken transport truck with inbuilt crates. The remaining space in the truck was also loaded with extra broilers to simulate natural transport conditions. The average temperature and relative humidity of the day was 37.5°C and 85%. The transport period for all six treatments was 8 hr. T₁ group (control group) was transported for 8 hr duration (without any lairage rest time). Treatment T₂ and T₃ were transported for 8 hour duration, and after the transport, broiler chickens were given 1 hour of lairage rest in normal lairage temperature and cooler lairage temperatures (20-25°C). Three days prior to transport last three treatment groups (T₄, T₅ and T₆) were supplemented with an antistressor and on 43rd day transported for duration of 8hr. After transport treatment groups T₅ and T₆ were given a 1 hr lairage rest under a normal lairage temperature and cooler lairage temperature (20-25°C) respectively. Transportation was on a country road from Bareilly towards Pilibhit. No feed or water was provided during and after transport. The broiler chickens were loaded off from their vehicles after transportation and they were assessed for their percentage change in body weight after transport and welfare parameters. They were slaughtered in the modern slaughter hall as per standard slaughtering techniques and guidelines and blood and muscle samples were collected (Total 10 samples from each treatment).

3.1.3 Preparation of Antistressor package:

Our main aim was to prepare a antistressor package that can have the capacity to reduce the adverse effects of transportation in Indian conditions. Previously at ICAR-CARI we have successfully worked on many antistressor/ antioxidant molecules individually. Based on studies and research done at CARI, we decided to choose the ingredients, that were tested at CARI in different experiments individually. We made antistressor package having four main ingredients of laboratory-grade products from the Sigma-Aldrich company. The dose level was decided based on the amount of water intake by 1 bird per day (generally a bird drinks 200-250 ml water per day during summer) and the other was dose of ingredient in water for birds. Before its use on large scale it was tested in the lab as well as in farm to check the palatability and side effects. But no adverse effects were seen on broilers and with this, we formulated final package preparation. Then it was mixed in the drinking water of broilers and

given three days before transport so as to curtail the stress faced by birds during transport and thus contributing in welfare and production improvement.

3.1.4 Assessment of Production parameters

1. Change in body weight

Total number of broilers in each treatment group (4 replicates with each replicates containing ten birds) were weighed together before transport. After transport, again these broilers were weighed to find out the absolute and percent change in body weight. The formula for calculation of absolute and percent change in body weight is as follows:

Absolute body weight change = Body weight of the birds in replicate before transport (kg) – Body weight of birds in replicate after transport (kg)

Percent change in body weight = Absolute body weight change (kg) / Total body weight (kg) per replicate $\times 100$

2. Assessment of Mortality percentage after transport

The number of broilers that died during transport and the total number of broilers transported in each treatment were counted and then mortality % was calculated according to the following formula:

% Mortality due to transport = Number of birds died due to transport / Total Number of birds transported $\times 100$

3.1.5. Assessment of Welfare Parameters

Gait score, Body temperature, Tonic Immobility (TI), Runaway test, Physical injuries, and Faecal score were examined to assess the welfare of the broilers.

- a) **Gait Score:** After transport walking ability of broilers was assessed by a three-point gait scoring system (Webster *et al.*, 2008) as mentioned in table 3.1 below.



Fig. 3.7: Tonic immobility test in broilers after transport



Fig. 3.8: Gait impairment in broilers after transport



Fig. 3.9: Runaway test in broilers after transport



Fig. 3.10: Physical injuries in broilers after transport



Fig. 3.11: Bloody/watery diarrhoea in broilers after transport

Gait Score	Degree of impairment	Criterion
0	None	Bird will walk for at least 5ft with a balanced gait. Bird may appear ungainly but with little effect on function.
1	Obvious impairment	Bird will walk for at least 5ft but with a clear limp or awkward gait
2	Severe impairment	Bird will not walk 5 ft. May shuffle on shanks or hocks with assistance of wings.

- b) Body temperature:** Rectal temperature of the broilers was recorded in degree Fahrenheit after transport by a (DR. MOREPEN Mt-100) digital thermometer.
- c) Tonic Immobility (TI):** Tonic immobility was assessed by Tonic immobility reaction time as mentioned by Ghareeb *et al.*, (2008). The broilers were kept on its back with gentle pressure on sternum and neck of the chickens for a period of 45 seconds. Then, the hand was removed gently and time was noted in the stopwatch till the chicken revert back to its normal position. This time period taken by the broilers to regain its normal position was used to access tonic immobility.
- d) Runaway (RA) test:** Runaway test was conducted as mentioned in Marin *et al.*, (2001). An iron corridor covered with gunny bags on the sides of the corridor was divided into three zones. The corridor opens with a start box and then was the stimulus bird zone. The other end of the corridor through which the chicken's runaway was littered with feed materials. The broilers were introduced into the start box and the time spent by the broilers in the stimulus bird zone was measured by an unobtrusive observer from behind during the 10 min test. The length, width and height of the corridor were 180cm, 40cm and 40cm respectively. Fifteen broilers from each treatment were assessed individually and the times taken for the broilers to move out of the corridor from the start box to end box were noted.
- e) Physical Injuries:** Physical condition were assessed by looking and clinical examination of broilers. The physical injuries observed after the transport includes bruises, fractures,

muscle injuries and hematoma. Total numbers of birds with and without physical injuries after transport in each treatment group were noted.

- f) Faecal score :** The faecal score was determined by 5 point scoring system in which 1 score was given to normal faeces and 5 was given to diarrheic faeces with blood (Ramadan *et al.*, 1997) as mentioned in table 3.2 below.

Score	Criterion
1	Normal faeces with solid consistency
2	Normal faeces with slight watery consistency
3	Semisolid consistency
4	Watery diarrhoea
5	Diarrheic faeces with blood

3.1.6. Assessment of Stress parameters

Serum corticosterone level and Heterophil/Lymphocyte ratio were analyzed to assess the transport stress levels in broiler chickens.

1. Estimation of Serum Corticosterone

In this present study, the serum Corticosterone values from collected serum samples were evaluated by competitive binding theory in Chicken Corticosterone ELISA kit of Bioassay Technology Laboratory (BT-LAB).

The underlying principle of this estimation is that microtiter wells are pre-coated with Chicken CORT antibody. CORT present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Chicken CORT Antibody is added and bind to CORT present in the sample. Then Streptavidin-HRP is added and binds to Biotinylated CORT antibody. After incubation unbound Streptavidin-HRP is washed away during washing. Substrate solution is then added and colour develops in proportion to amount of chicken CORT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

2. Assessment of Heterophil/Lymphocyte ratio (H/L ratio)

Thin blood smears were made from the blood collected (from wing vein by using a sterile niddle) before slaughter of the birds using microscope glass slides. The blood smear slides were air dried and fixed with absolute alcohol. Then smears were stained by Giemsa's staining as mentioned in the procedure of HiMedia's giemsa staining kit. After staining, the slides were air dried and examined under 100x magnification using one drop of cedar wood oil. To determine the *heterophil* and *lymphocyte* count, a minimum of 100 cells per field were examined by light microscopy (Ghareeb *et al.*, 2008).

3.1.7. Assessment of Gene Expression of HSP-70 gene by Real-time PCR

The major emphasis of this study was to assess the effect of anti-stressor agents on transportation stress and mRNA expression of the HSP-70 gene of broiler chickens after transport. The expression profile of the HSP-70 gene was assessed by using real-time polymerase chain reaction (QRT-PCR).

The following procedure was employed for the purpose

- a. Collection of tissue (jejunum) samples
- b. Preparation of samples
- c. Total RNA isolation from tissues
- d. Reverse Transcriptase-PCR for first strand cDNA synthesis
- e. PCR amplification and quantification by Real-time PCR.

Sample collection and storage

Four chicks (2 males and 2 female) from each treatment were randomly selected and sacrificed at 43rd days after transport for sampling. About 50 mg of jejunum samples were dissected out from each broiler. The samples were collected aseptically, placed immediately in labeled DEPC-treated and autoclaved in 1.5 ml micro-centrifuge tubes containing 1 ml of TRIzol reagent solution. The tissue samples were kept at 4° C and then stored at -20° C until processed for RNA extraction. All sampling procedures were performed on a clean laboratory condition with surfaces and instruments (scissors, scalpels and forceps) sterilized with diethyl

pyrocarbonate (DEPC). The tissues were then homogenized to disrupt the cells using automated homogenizer (Polytron) on high setting for 30-60 seconds or until no tissue fragments or clumps were visible. Then the samples were processed to isolate total RNA by Trizol method.

Total RNA Isolation from jejunum tissues by TRIZOL method

Homogenization of jejunum tissue

RNA from jejunum tissues was isolated by TRIZOL method (Invitrogen, Thermo Fischer Scientific, USA) as per the manufacturer's specifications. Briefly, 50 mg of jejunum tissues was taken in 1 ml of TRIZOL reagent and homogenized until the clear suspension was obtained, and transferred into Eppendorf tubes. The suspension was incubated for 10 min at RT (for dissociation of nucleoprotein).

Phase separation

After homogenization, 200 µl of chloroform solution was added and mixed vigorously for 15 seconds and incubated for 10 minutes in ice. Then the samples were centrifuged at 12000 rpm for 15 minutes at 4 °C for phase separation. After centrifugation, 3 separate layers were formed, the upper aqueous phase, the middle interphase and the lower protein Trizol phase.

RNA precipitation

The aqueous colourless phase was collected into a new Eppendorf tube and to that 500 µl (per ml of TRIZOL reagent) of isopropanol was added. The samples were incubated at 15-30 °C for 10 minutes and centrifuged at 12000 rpm for 15 min at 4 °C.

RNA washing

The supernatant was discarded and the RNA pellet at the bottom was washed with 70% ethanol. The ethanol was discarded after centrifugation at 12000 rpm for 10 min at 4 °C.

Drying

The RNA pellet was air dried in laminar flow, dissolved in 25-50 µl of nuclease free water according to the pellet size, and stored at -20 °C for complementary DNA (cDNA) synthesis.

Determination of purity and concentration of RNA

The concentrations and purities of RNA preparations were determined spectrophotometrically taking absorbance at 260 and 280 nm by Nanodrop instrument. The A260/A280 ratio of the samples was greater than 1.8. The possible traces of genomic DNA were removed by treating 5 µg of each RNA samples with 5 IU of RNase-free DNase at 37°C for 1 hr. The DNase was subsequently inactivated by incubation at 65°C for 10 min. Samples were stored at –80°C for cDNA synthesis.

First-strand cDNA synthesis by RT-PCR:

After taking the O.D. at 260/280, the concentration of each RNA sample was computed and all the samples were adjusted to a uniform concentration (5000 ng/20 µl). The required amount of RNA from each sample was taken as template and the first strand cDNA was prepared by Thermo Scientific Verso cDNA synthesis kit (Catalogue No. AB1453/A) using random hexamer primers. Following reaction mixture was prepared in a tube on ice given in table 3.3 below.

	Volume	Final Concentration
5X cDNA synthesis buffer	4 µL	1X
dNTP Mix	2 µL	500 µM each
RNA Primer*	1 µL	
RT Enhancer	1 µL	
Verso Enzyme Mix	1 µL	
Template (RNA)	1-5 µL	1 ng
Water, nuclease-free	upto 20 µL	
Total volume	20 µL	

Table 3.4 Reverse transcription cycling program

	Temperature	Time	Number of cycles
cDNA synthesis	42 °C	30 min	1 cycle
Inactivation	95 °C	2 min	1 cycle

Designing and standardization of primer for PCR

Anti-sense and sense-specific oligonucleotide primers shown in Table 3.5 were designed to amplify the HSP 70 in jejunum tissue samples. The primers were standardized for the PCR reaction conditions. The amplification was carried out with a total of 25 µl volume, containing 10 pmole of each primer, 0.2mmole dNTP mix, 1 unit of Taq DNA polymerase (Fermentas, USA.) and 1 µl cDNA in 5x Taq polymerase buffer. PCR cycling conditions were: initial denaturation at 94°C (5 min); 36 cycles of denaturation at 94°C (30 s), annealing temperature as mentioned in Table 3.5 for 30 sec and extension at 72°C (30 sec) followed by final extension at 72°C (15 min). The sizes and quantity of PCR products were verified by comparison with a DNA ladder. The samples yielded products of the expected sizes following PCR with gene-specific primers.

Table 3.5 Details of primer and annealing temperatures used in PCR assay

Sl No.	Gene	Primer	Annealing temperature	Length	Reference/ Acc. Number
1	HSP-70	F-GGCCGCAAGTATGATGACCCCA R-GGCTTGCCACCCTCGTTCACCAC	60 °C	80bp	-
2	28S	F-CAGGTGCAGATCTTGGTGGTAGTA R-GCTCCCGCTGGCTTCTCC	60 °C	273bp	JN639848

Setting up of PCR reaction

For setting up of PCR (n+1), 200 µl PCR tubes were taken (where n=No. of samples) and were numbered. At first 1 µl of the above cDNA containing approximately 100ng was added to each PCR tube and kept on ice. In a 1.5 ml microcentrifuge tube (n+1) x 22 µl of master mix and Taq polymerase 3U/ml @1U/sample was added and volume was made up to (n+1) x 24 µl using autoclaved Milli-Q water. Finally, 24 µl of this cocktail was added and each PCR tube was mixed by tapping and spinning. A negative control (devoid of the template but complete in all aspects) was also used to check the PCR carried out. Finally, the PCR tubes were kept in a thermocycler. Cycling conditions included, initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 30 secs, annealing at temperature as standardized earlier, extension at 72°C for 30sec, and a final extension at 72°C for 15 min.

Agarose gel electrophoresis

The Amplified fragments were separated by gel electrophoresis in a 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide in TAE (1x) buffer for 1 hour at constant voltage (100V). After electrophoresis, the gel was examined under UV transilluminator and photographed for documentation.

Differential expression of the HSP-70 gene

Quantification of mRNA expression of the HSP-70 gene in jejunum tissues from 4 birds (2 males and 2 female) for each treatment at 42nd day post hatch was done by relative quantitative method real-time PCR (Q-PCR) involving mRNA expression level of a house keeping gene (28s) as control.

Quantitative SYBR green polymerase chain reaction (PCR) assay

Quantitative SYBR Green PCR assay on jejunum samples were performed using Thermal cycler (Qiagen, Germany) and commercial reagents from DyNAmo Flash SYBER green qPCR kit (F-416L) as per manufacturer's recommendations. PCR reaction mixture comprised of 2x SYBR Green PCR mix, 10 pmoles each of sense and anti-sense primers, 1 µl cDNA and nuclease free water to make up total reaction volume of 20 µl. The reaction mixture was mixed by vortexing, centrifuged and transferred to optical-grade strip capped 200 µl PCR tubes. Reactions were set in triplicates for each treatment for SYBR Green PCR and light exposure was minimized/avoided while set up.

The thermal profile is comprised of three segments. Segment 1 comprised of initial denaturation at 94°C for 5 minutes followed by segment 2 consisting of 40 repetitive cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 45 secs each (Table 3.6). Dissociation curve analysis was performed in segment 3, which comprised of a single cycle of denaturation at 95°C for 1 minute, partial denaturation to 55-59°C for 30 seconds, and denaturation again at 95°C for 30 seconds. For generation of amplification plots, fluorescence values (dR) were collected at second plateau (annealing phase) and to confirm fidelity of amplification, dissociation curve analysis was performed at the end of the run. The reactions were performed according to the following temperature and cycle.

Table 3.6 The number of cycles, time and temperature used for real time assay

Time	Temperature	Cycles
5 minutes	94 °C	1
30 seconds	94°C	40
30 seconds	60°C	
30 seconds	72°C	

Analysis of real time PCR results

For gene expression studies of HSP70 mRNA in relation to reference gene real-time PCR data was analysed by using **REST-2009** software package.

3.1.8 Assessment of meat characteristics

Meat characteristics like pH value, Shear Force Value (SFV), Water holding capacity (WHC), drip loss of meat, and ABTS were examined to study the effect of transport on the meat quality of the broilers. A total of ten breast meat samples from ten broilers in each treatment were collected after slaughter.

a) pH

The pH of meat sample was measured by method devised by Troutt *et al.* (1992). The pH meter was calibrated by using a pH buffer solution (pH 4.0 and 9). Five gm of meat sample were blended with 25 ml of distilled water for 2 min using pestle and mortar. The pH of the homogenate was recorded by immersing the combined glass electrode of a digital pH meter in it. The electrode was wiped again with distilled water and fixed in the buffer solution.

b) Shear force value

SFV was analysed to determine the tenderness of the meat. Meat samples were stored at a temperature of -20°C for overnight period and thawed to room temperature before they were analysed for tenderness by Warner-Bratzler shear force protocol as mentioned in Berry and Stiffler, (1981). The meat samples were cut into uniform cylindrical shaped meat cores of 1cm diameter. In this protocol, the V shaped Warner-Bratzler shear blade were used to cut

perpendicularly the longitudinal muscle fibres of the meat. This cut in the meat sample gives the tenderness of the meat with the resultant values in kg/cm².

c) Water holding capacity

WHC is defined as the ability of meat to retain its water during the application of external force such as cutting, grinding, pressing, heating, etc. WHC of the muscle tissue has a direct effect on its shrinkage during storage. The WHC of meat samples was determined by the method devised by Wardlaw *et al.* (1973).

Procedure-

- Take 50 ml polycarbonate centrifuge tube and put 10 g of finely minced meat samples in it.
- Add 15 ml of 0.6 M NaCl solution to it and mix for 2 minutes.
- Hold for 15 min at 4°C in a refrigerator in order to allow the effect of NaCl and phosphate to reach equilibrium.
- Shake the slurry and centrifuge at 5000 rpm for 15 min. in a refrigerated centrifuge.
- Decant the supernatant fluid in a measuring cylinder, measure and record it as V.

$$\text{WHC (\%)} = \frac{\text{Volume of NaCl added} - \text{Volume of supernatant}}{\text{Weight of sample}} \times 100$$

d) Drip loss

The frozen meat sample from the thigh and breast were taken and weighed and recorded as the initial weight (W₁). The weighed sample were placed into a polyethylene plastic bag, labelled, and stored hanging at 4°C for 24 hr. the samples were weighed again and recorded as final weight (W₂). Drip loss was calculated as the difference between the initial and final weight of the sample and expressed as the percentage of the initial weight of the sample as shown in the equation below.

$$\text{Drip loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

W₁ = Initial weight of meat sample

W₂ = Final weight of sample (after 24 hours)

e) ABTS assay

The spectrophotometric analysis of ABTS⁺ radical scavenging activity of both breast and thigh muscle samples was determined according to the method of Shirwaekar *et al.* (2006). This method was based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734nm, in comparison to that of standard antioxidant. ABTS⁺ was dissolved in water to a 7mM concentration. ABTS radical cation (ABTS⁺) were produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (K₂S₂O₈) and allowing the mixture to stand in the dark room at ambient temperature for 16 hours before use, because ABTS and potassium persulphate react stoichiometrically in the ratio of 1:0.5 resulting in incomplete oxidation of ABTS⁺, oxidation of ABTS⁺ commenced immediately, but the absorbance was not maximal and stable until 6 hours elapsed. The radical was stable in this form for more than 2 days, when stored in the dark at ambient temperature. Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.7 and equilibrated at 30°C exactly 6 minutes after initial mixing to make working solution of ABTS.

Procedure

In this method 5g meat sample was triturated in 20 ml of ethanol for 2 minutes with the help of pestle and mortar. The homogenate was quantitatively transferred to a beaker and filtered through Whatman filter paper No.42. then 2 ml of ABTS working solution was mixed with 1 ml of filtrate prepared and absorbency was measured after 20 minutes (t₂₀) at 734nm. The ABTS activity was calculated by using the formula:

$$\text{ABTS activity (\% inhibition)} = \frac{(0.7 - A_{t_{20}})}{3.1 \times 100}$$

3.1.9 Assessment of Carcass traits

After transport 10 birds from each treatment were randomly selected and slaughtered for evaluation of carcass characteristic and organ weight.

1. Dressing % = $\frac{\text{Weight of dressed bird with giblet (g)}}{\text{Pre slaughter live weight (g)}} \times 100$
2. Eviscerated yield% = $\frac{\text{Weight of eviscerated bird without giblet (g)}}{\text{Pre slaughter live weight (g)}} \times 100$
3. Heart % = $\frac{\text{Weight of heart (g)}}{\text{Pre slaughter live weight (g)}} \times 100$
4. Gizzard % = $\frac{\text{Weight of gizzard (g)}}{\text{Pre slaughter live weight (g)}} \times 100$
5. Liver % = $\frac{\text{Weight of liver (g)}}{\text{Pre slaughter live weight (g)}} \times 100$
6. Giblet% = $\frac{\text{Weight of heart+ gizzard+ liver (g)}}{\text{Pre slaughter live weight (g)}} \times 100$
7. Part yield % = $\frac{\text{Weight of part (breast / thighs / wings / drumstick)}}{\text{Pre slaughter live weight (g)}} \times 100$

3.2.0 Assessment of serum biochemical parameters

The blood samples were collected from the jugular vein by puncturing vein with 24G middle with least stress to the birds, in sterile serum vials after end of transport. Total ten birds from each treatment making a total of 60 samples. The serum was separated after keeping the vial at slanting position for an hour, and then centrifuged in the lab at 3000rpm for 10min. After centrifugation serum samples were transferred into a separate eppendorf tubes and were labelled and stored at -20°C for further biochemical analysis.

a) Estimation of serum cholesterol

In the present study, serum cholesterol values of collected samples were evaluated by 'PAP' method with the use of standard kit procured from the Coral Clinical Systems, India.

The underlying principle present in this method is that cholesterol esterase hydrolyses esterified cholesterol to free cholesterol which is oxidised to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form

a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

Procedure

Test tubes were labelled as B, for blank; S for standard and T, for test.

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Cholesterol standard	-	0.01	-
Sample	-	-	0.01

The mentioned contents were mixed accordingly and incubated at 37°C for 5min. Thereafter, by using the spectrophotometer the absorbance of standard (S) and test (T) were measured against the blank (B) within 60 min at 505nm.

$$\text{Serum cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 200$$

b) Estimation of serum uric acid

In this present study, the serum uric acid values from collected sample were evaluated by 'Uricase/PAP' method with the use of standard kit procured from the Coral Clinical Systems, India.

The underlying principle present in this method is that uricase converts uric acid to allantoin and hydrogen peroxide. The hydrogen peroxide further reacts with a phenolic compound and 4-aminoantipyrine to form a red coloured dye complex. Intensity of the colour formed is directly proportional to the amount of uric acid present in the sample.

Procedure

Test tubes were labelled as Blank (B), Standard (S) and Test (T).

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
Working reagent	1.0	1.0	1.0
Distilled water	0.02	-	-
Uric acid standard	-	0.02	-
Sample	-	-	0.02

The mentioned contents were mixed accordingly and incubated at 37°C for 5 min. Thereafter, by using the spectrophotometer the absorbance of standard (S) and test (T) were measured against the blank (B) within 30 min at 520 nm.

$$\text{Uric acid in mg/dl} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 8$$

c) Estimation of serum creatinine

In this present study, the serum creatinine values from collected samples were evaluated by 'Modified Jaffe's Kinetic method' method with the use of standard kit procured from the Coral Clinical Systems, India.

The underlying principle in this method is that picric acid in alkaline medium reacts with creatinine to form an orange coloured complex with alkaline picrate. The intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Procedure:

Test tubes were labelled as Test (T) and Standard (S)

Addition sequence	Standard (ml)	Test (ml)
Picric Acid Reagent (L1)	0.5 ml	0.5ml
Buffer Reagent (L2)	0.5ml	0.5ml
Bring reagents to the assay temperature (S-30°C/ B-37°C) and add		
Creatinine standard/sample	0.1ml	0.1ml

The contents were mixed well and the initial absorbance A_1 for the Standard and Test were read exactly after 30 seconds at 520nm. Another absorbance A_2 of the Standard and Test were read exactly after 60 seconds later at 520nm. Change in absorbance ΔA for both the 'S' and 'T' were calculated.

$$\text{For Standard } \Delta AS = A_2S - A_1S$$

$$\text{For Test } \Delta AT = A_2T - A_1T$$

$$\text{Creatinine in mg/dl} = \frac{\Delta AT}{\Delta AS} \times 2$$

d) Estimation of Alanine Amino Transferase (ALAT/SGPT)

In this present study, the serum ALAT values from collected samples were evaluated by 'Modified IFCC' method with the use of standard kit procured from the Coral Clinical Systems, India.

The underlying principle in this method is that SGPT catalyzes the transfer of amino group between L-Alanine and α -ketoglutarate to pyruvate and glutamate. This pyruvate reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as decrease in absorbance which is proportional to the SGPT activity in the sample.

Procedure

Test tubes were labelled as Blank (B) and Test (T)

Addition Sequence	Test (ml) 25°C /30°C	Test (ml) 37°C
Enzyme Reagent (L1)	0.8	0.8
Sample	0.2	0.1
Incubate at assay temperature for 1 min and add		
Starter Reagent (L2)	0.5	0.5

The contents were mixed and initial absorbance A_0 were read after 1 min at 340nm. The absorbance reading was repeated after every 1, 2 & 3 min. Mean absorbance was calculated as change per min ($\Delta A/\text{min}$).

$$\text{SGPT Activity in U/L (25°C/30°C)} = \Delta A/\text{min.} \times 952$$

$$\text{SGPT Activity in U/L (37°C)} = \Delta A/\text{min} \times 1746$$

e) Estimation of Aspartate Amino Transferase (ASAT/SGOT)

In this present study, the serum ASAT values from collected samples were evaluated by 'Modified IFCC' method with the use of standard kit procured from the Coral Clinical Systems, India.

The underlying principle in this method is that SGOT catalyzes the transfer of amino group between L-Aspartate and α -ketoglutarate to form Oxaloacetate and Glutamate. This oxaloacetate reacts with NADH in the presence of Malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as decrease in absorbance which is proportional to the SGOT activity in the sample.

Procedure:

Test tubes were labelled as Blank (B) and Test (T)

Addition Sequence	Test (ml) 25°C /30°C	Test (ml) 37°C
Enzyme Reagent (L1)	0.8	0.8
Sample	0.2	0.1
Incubate at the assay temp for 1 min. and add		
Starter Reagent (L2)	0.2	0.2

The contents were mixed and initial absorbance A_0 were read after 1 min at 340nm. The absorbance reading was repeated after every 1, 2 & 3 min. Mean absorbance was calculated as change per min ($\Delta A/\text{min}$).

$$\text{SGPT Activity in U/L (25°C/30°C)} = \Delta A/\text{min.} \times 952$$

$$\text{SGPT Activity in U/L (37°C)} = \Delta A/\text{min} \times 1746$$

f) Estimation of Total Protein

In this present study, the total protein values from collected samples were evaluated by 'Biuret method' method with the use of standard kit procured from the Coral Clinical Systems, India.

The underlying principle in this method is that protein in alkaline medium reacts with cupric ions (present in biuret reagent) to form a blue-violet-colored complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

Procedure

Test tubes were labelled as Blank (B), Standard (S) and Test (T).

Addition sequence	Blank (ml)	Standard (ml)	Test (ml)
Biuret reagent (L1)	1.0	1.0	1.0
Distilled water	0.02	-	-
Protein standard (S)	-	0.02	-
Sample	-	-	0.02

The mentioned contents were mixed accordingly and incubated at 37°C for 10 min or at R.T. for 30 min. Thereafter, by using the spectrophotometer the absorbance of standard (S) and test (T) were measured against the blank (B) within 60 min at 550 nm.

$$\text{Total Protein in mg/dl} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 8$$

Statistical Analysis

- ❖ Data generated during the course of study was statistically analysed by two-way ANOVA test using SPSS software.
- ❖ Significance different between means was estimated by DMRT test (1955).



Results



This research work was carried out at the Division of Poultry housing and Management, ICAR-Central Avian Research Institute, Izatnagar, Bareilly-243122 (UP). With the aim of **Amelioration of transportation stress by antistressor package supplementation and lairage environment for better meat quality and welfare of broiler chicken during summer**. In the present trial, Two Hundred and forty broiler chickens of 42 days old were divided into six treatment groups with each group consisting of forty broilers. The broiler chickens were transported in medium-sized chicken transport trucks with in-built crates, also birds were painted with different colour as per treatment for identification. The remaining space is also filled with extra birds so that natural transport scenario was created. The temperature and relative humidity on the day of transportation of the trial was 37.5°C and 85 %. The factorial design was followed. Based on the results of the trial, the effect of the antistressor and lairage environments on broiler chickens was determined.

Objective: To evaluate the effect of antistressor package supplementation lairage and environments on production, welfare, HSP-70 gene expression, meat quality and physio-biochemical attributes in transport stressed broiler chickens

The effect of antistressor package supplementation and lairage environment after transport on production, welfare, HSP-70 gene expression, meat quality and physiobiochemical attributes were assessed and are depicted below. The broiler chickens in all six treatments were transported for 8 hour duration, in which T₁ was kept as control and was slaughtered without any lairage rest, and treatments T₂ and T₃ were given 1-hour lairage rest in normal

lairage temperature and cooler environment respectively. Before the start of transport, the last 3 treatments (T_4 , T_5 and T_6) were supplemented with an antistressor given to them 72 hrs before the start of transport through drinking water. Treatments 5 and 6 were given 1 hr lairage rest in normal and cooler lairage environment respectively.

4.1. Production parameters

4.1.1. Body weight change

The result of the effect of antistressor package supplementation and lairage environments on absolute and percentage body weight in broiler chickens is given in Table 4.1. Supplementation of antistressor package before transport significantly ($P<0.05$) affected absolute and percentage body weight change in broiler chickens in the treatment groups. The percentage body weight change in broiler chickens in supplemented group and in the non-supplemented group was -7.13% and -8.66% respectively. The different lairage environment also had significant ($P<0.05$) impact on the percentage body weight change in broiler chickens and the percentage body weight change in broiler chickens was found less in cooler lairage temperature group which was -7.292% as compared to normal lairage temperature group which was -7.904% and control group was -8.502%. The interaction of antistressor package supplementation and lairage environment had significant ($P<0.05$) impact on the percentage and absolute body weight change in broiler chickens. Percentage body weight change in broiler chickens was found lower in Treatment group T_6 which was -6.37% and higher in T_1 which was -9.254%.

4.1.1. Mortality percentage

In the present study, no mortality was seen in all six treatment groups. However significant injuries were seen in non-supplemented group.

4.2 Welfare parameters

The result of the effect of antistressor package supplementation and lairage environments on welfare parameters in broiler chickens is presented in Table 4.2.

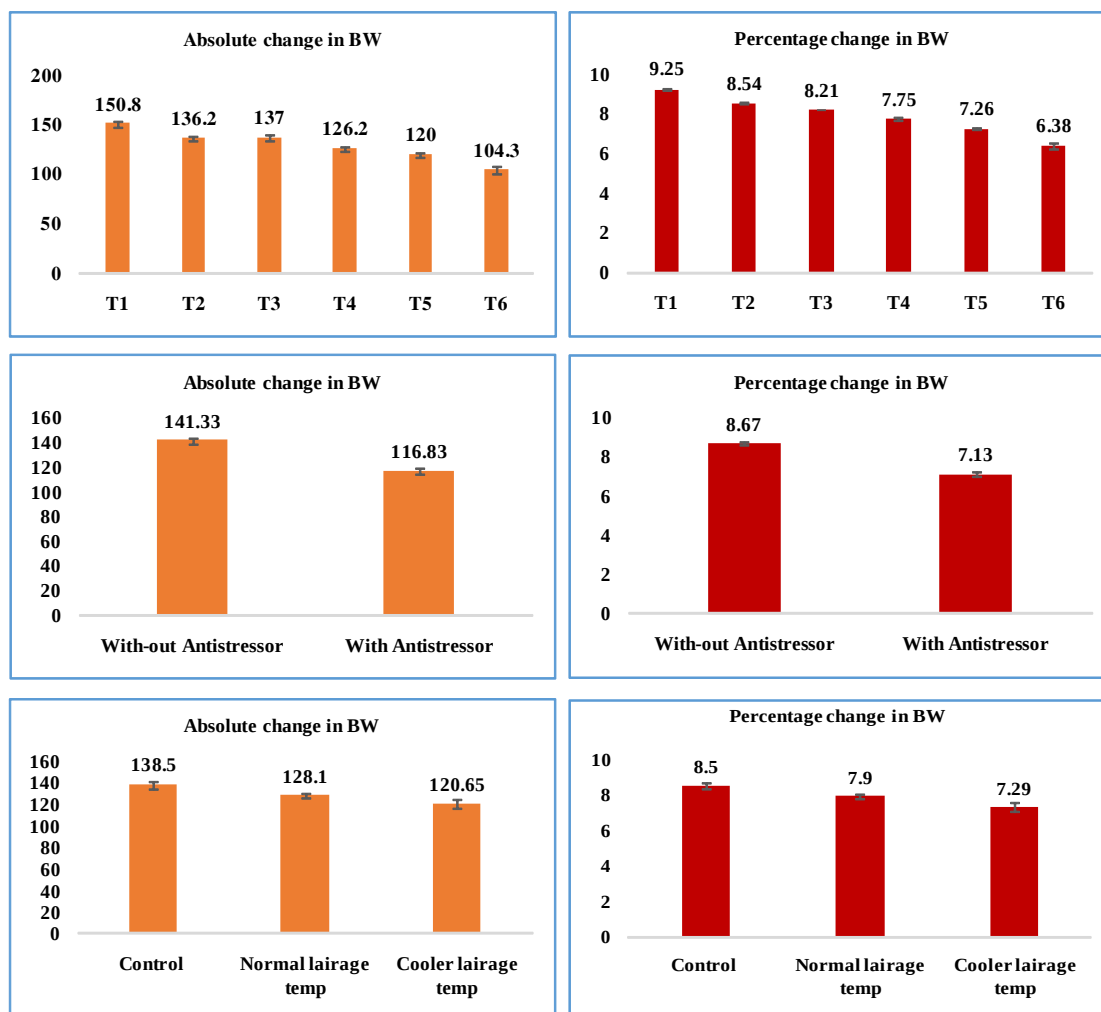


Fig. 4.1: Graphical representation of effect of antistressor package supplementation and lairage environment on absolute and percentage change in Body weight

Table 4.1 Effect of antistressor package supplementation and lairage environment on Body weight

Treatment Grp.	BW before transport	BW after transport	Absolute change in BW	% change in BW
Anti-stressor x condition				
T1	1631.00±35.94	1480.20±33.29	150.80 ^d ±2.74	9.2549 ^f ±0.06
T2	1594.40±26.39	1458.20±24.18	136.20 ^c ±2.27	8.5426 ^e ±0.03
T3	1669.40±45.96	1532.40±42.35	137.00 ^c ±3.64	8.2091 ^d ±0.03
T4	1629.20±30.51	1503.00±28.38	126.20 ^b ±2.32	7.7484 ^c ±0.05
T5	1652.10±35.82	1532.10±33.34	120.00 ^b ±2.53	7.2648 ^b ±0.03
T6	1634.50±47.36	1530.20±44.05	104.30 ^a ±4.18	6.3756 ^a ±0.16
P value	0.467	0.545	0.031	0.002
Effect of antistressor				
Without antistressor	1631.60±21.36	1490.27±19.85	141.33 ^b ±2.05	8.669 ^b ±0.08
With Antistressor	1638.60±21.54	1521.77±20.13	116.83 ^a ±2.44	7.130 ^a ±0.12
P value	0.821	0.275	0.001	0.001
Effect of lairage environment				
Control	1630.10±22.94	1491.60±21.45	138.50 ^b ±3.32	8.502 ^c ±0.18
Normal lairage temp	1623.25±22.64	1495.15±21.76	128.10 ^{ab} ±2.49	7.904 ^b ±0.15
Cooler lairage temp	1651.95±32.36	1531.30±29.74	120.65 ^a ±4.62	7.292 ^a ±0.22
P value	0.731	0.460	0.001	0.001

[†]Percentage change in body weight is the mean change in a total of forty birds in four replicates.

[‡]The percent change in body weight was Arsin transformed before statistical analysis.

n=10; Mean bearing different superscripts (a-f) superscript row-wise differ significantly (P<0.05).

4.2.1. Gait score

Supplementation of antistressor package before transport significantly (P<0.05) affected the gait score in the treatment groups. The degree of gait impairment was decreased in the supplemented group which was 1.067 as compared to the non-supplemented group which was 0.83. The effect of the lairage environment significantly affected (P<0.05) the gait score and the gait score was found less in normal lairage temperature group (0.45) compared to the cooler lairage temperature group (0.65) and control group (1.75). Under the interaction (between antistressor package supplementation and lairage environment) the gait score was significantly (P<0.05) improved. Gait score was found more in treatment group T₁ which was 2.30 and least in T₂ (0.10) and in treatment group T₆ (0.50).

Table 4.2 Effect of antistressor package supplementation and lairage environment on welfare Parameters

Treatment Grp.	Tonic Immobility	Run away test	Gait Pattern	Injury	Faecal test	Temp
Anti-stressor x condition						
T1	148.8±37.91	6.90 ^c ±0.31	2.30 ^b ±0.37	0.10±0.10	2.3±0.95	109.23 ^d ±0.49
T2	112.123.62	5.20 ^b ±0.20	0.10 ^a ±0.10	0.10±0.10	1.5±0.85	108.75 ^d ±0.23
T3	64±23.96	4.50 ^b ±0.17	0.80 ^a ±0.29	0.30±0.15	1.1±0.32	107.38 ^c ±0.17
T4	40±25.68	1.53 ^b ±0.53	1.20 ^{ab} ±0.36	0.00±0.00	1.9±0.57	106.90 ^{bc} ±0.40
T5	1.4±0.22	1.16 ^a ±0.21	0.80 ^a ±0.20	0.50±0.34	1.4±0.52	105.08 ^a ±0.20
T6	1.2±0.13	1.33 ^a ±0.16	0.50 ^a ±0.22	0.00±0.00	1.0±0.00	105.68 ^{ab} ±0.19
P value	0.512	0.002	0.007	0.097	0.679	0.008
Effect of antistressor						
Without antistressor	108.3 ^b ±17.53	5.53 ^b ±0.23	1.067±0.23	0.167±0.07	1.633±0.16	108.45 ^b ±0.24
With Antistressor	14.333 ^a ±8.94	1.33 ^a ±0.19	0.8330.16	0.1670.12	1.4330.10	105.89 ^a ±0.22
P value	0.001	0.001	0.30	1.00	0.217	0.001
Effect of lairage environment						
Control	94.60 ^b ±25.51	4.21 ^b ±0.68	1.75 ^b ±0.28	0.05±0.05	2.1 ^b ±0.18	108.06 ^b ±0.42
Normal lairage temp	56.75 ^{ab} ±17.13	3.18 ^a ±0.48	0.45 ^a ±0.13	0.30±0.18	1.45 ^a ±0.15	106.91 ^a ±0.49
Cooler lairage temp	32.60 ^a ±13.71	2.90 ^a ±0.38	0.65 ^a ±0.18	0.15±0.08	1.05 ^a ±0.05	106.53 ^a ±0.25
P value	0.033	0.001	0.001	0.313	0.001	0.001

n=15; Mean bearing different small letter (a-d) superscript row-wise differ significantly (P<0.05)

4.2.2. Body temperature

Supplementation of antistressor package before transport significantly (P<0.05) affected the body temperature in the treatment groups. Body temperature was decreased in the supplemented group (105.89°F) as compared to the non-supplemented group which had a temperature of 108.45 °F. The effect different lairage environment was also found significant (P<0.05) on body temperature and the body temperature was found less in the cooler lairage temperature group i.e. 106.53 °F as compared to normal lairage temperature group having 106.91 °F and control group having temperature of 108.06 °F. The interaction of antistressor package supplementation and lairage environment also had significant (P<0.05) impact on the body temperature. Treatment group T₁ recorded a maximum body temperature which was 109.23°F and T₅ had a minimum body temperature which was found to be 105.08 °F.

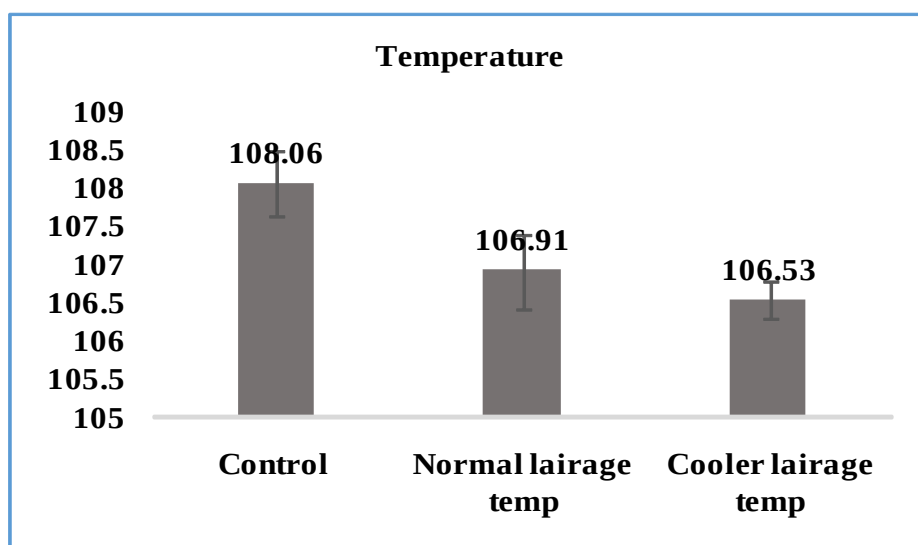
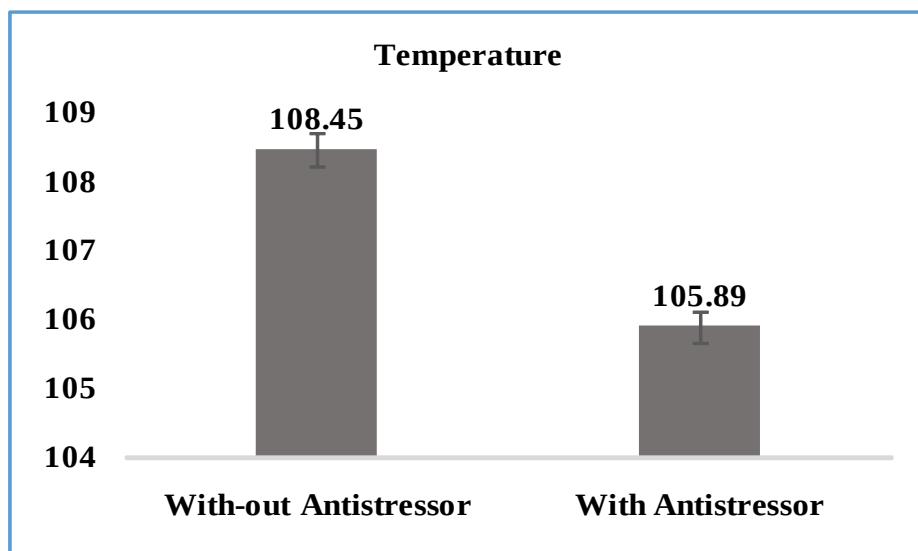
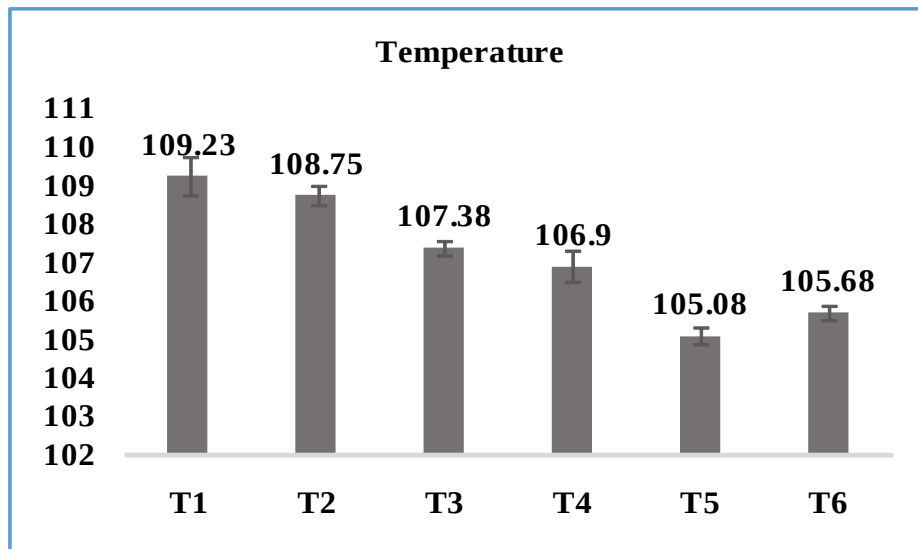


Fig. 4.2: Graphical representation of effect of antistressor package supplementation and lairage environment on welfare parameters

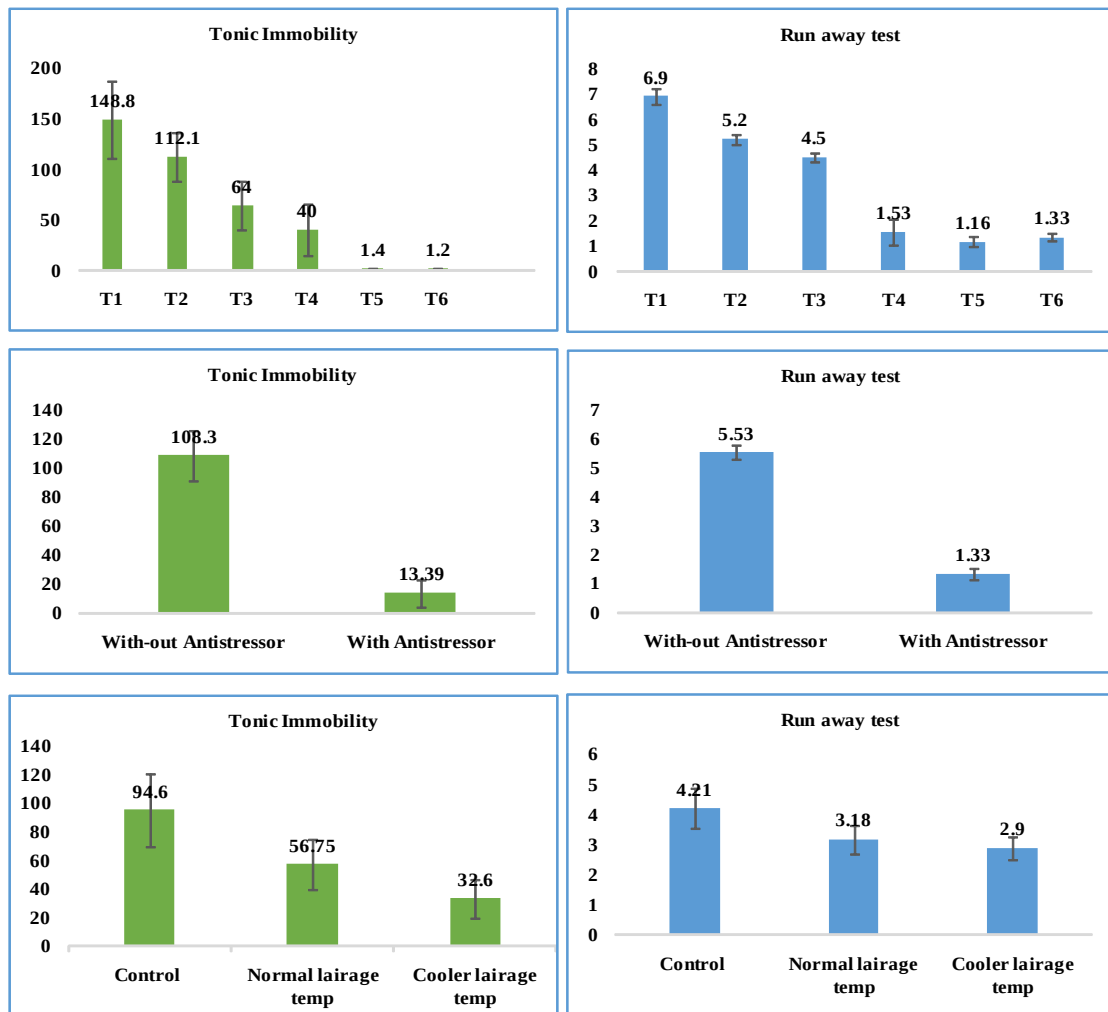


Fig.4.2 Contd...

4.2.3. Runaway test

Supplementation of antistressor package before transport significantly ($P < 0.05$) affected the runaway test in the treatment groups. Value of the runaway test was decreased in the supplemented group i.e. 1.33 as compared to the non-supplemented group having 5.53. The effect of different lairage environment was found significant ($P < 0.05$) on runaway test and the runaway test was found less in cooler lairage temperature group (2.90) as compared to normal lairage temperature group (3.18), and control group (4.21). The interaction of the antistressor package supplementation and lairage environment had significant ($P < 0.05$) impact on the runaway test in treatment groups and treatment group T_1 recorded a maximum runaway test of 6.90 and T_6 had minimum runaway test of 1.33.

4.2.4. Tonic immobility (TI)

Antistressor package supplementation had a significant ($P < 0.05$) impact on the tonic immobility activity of broilers in the treatment groups. Broilers in the supplemented group showed less TI period which was of 14.33 as compared to non-supplemented group. Whereas, broilers in cooler lairage temperatures group showed lower TI period which was of 32.60 sec when compared to other treatment groups (normal lairage temperature and control) and this decrease in value was not significant ($P > 0.05$). The interaction of antistressor package supplementation and lairage environment linearly decreased the tonic immobility in the treatment groups but was found non-significant ($P > 0.05$). The value tonic immobility period was highest in T_1 group and lower in T_6 group.

4.2.5. Physical injuries

Antistressor package supplementation, lairage environment, interaction of antistressor package supplementation and lairage environment had no significant ($P > 0.05$) impact on injuries. However, the values under interaction were lowest in T_4 (0), and T_6 (0) group and highest in T_5 (0.05). In different lairage environment, the injury was less in control group (0.05), as compared to cooler lairage temperature group (0.15) and normal lairage temperature environment group (0.30).

4.2.6. Faecal test

Lairage environment had significant ($P<0.05$) impact on the faecal score. The faecal score was 1.05, 1.45, and 2.1 in cooler lairage temp group, normal lairage temp group, and control lairage environment respectively. Antistressor package supplementation and interaction of antistressor package supplementation and lairage environment had no significant ($P>0.05$) impact on the faecal score. However, the faecal score was less in supplemented group (1.43) as compared to the non-supplemented group (1.63). In the interaction of antistressor and lairage environment, faecal score was lowest in T₆ (1.0) and highest in T₁ (2.3).

4.3 Stress parameters

The result of the effect of antistressor package supplementation and lairage environments on serum corticosterone level and Heterophil/Lymphocyte (H/L) ratios in broiler chickens is shown in Table 4.3.

Table 4.3 Effect of antistressor package supplementation and lairage environment on stress parameters

Treatment Grp.	H/L Ratio	Corticosterone ($\mu\text{g/L}$)
Anti-stressor x condition		
T1	1.45 ^a ±0.02	172.922 ^c ±3.21
T2	1.21 ^d ±0.02	151.471 ^b ±2.31
T3	0.86 ^c ±0.01	138.025 ^{ab} ±1.68
T4	0.56 ^b ±0.004	128.529 ^a ±0.75
T5	0.51 ^b ±0.002	129.907 ^a ±5.54
T6	0.39 ^a ±0.002	125.858 ^{ab} ±2.76
P value	0.001	0.001
Effect of antistressor		
Without antistressor	1.17 ^b ±0.05	154.140 ^b ±4.06
With Antistressor	0.49 ^a ±0.01	133.098 ^a ±2.42
P value	0.001	0.001
Effect of lairage environment		
Control	1.004 ^c ±0.12	150.726 ^b ±7.56
Normal lairage temp	0.858 ^b ±0.09	140.689 ^a ±4.57
Cooler lairage temp	0.623 ^a ±0.06	139.442 ^a ±1.60
P value	0.001	0.001

n=10; Mean bearing different small letter (a-e) superscript row-wise differ significantly ($P<0.05$).

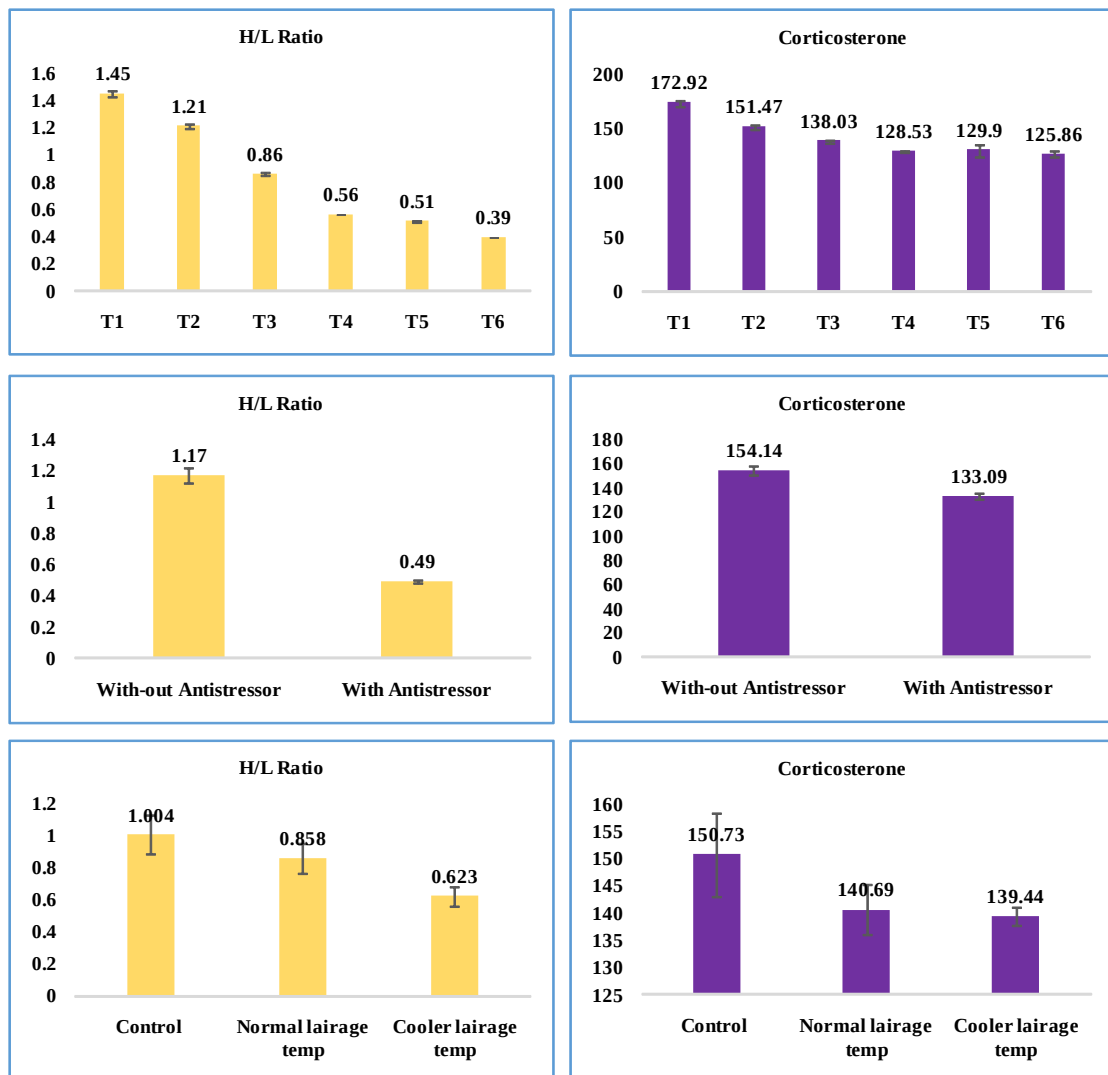


Fig. 4.3: Graphical representation of effect of antistressor package supplementation and lairage environment on stress indicators

4.3.1. Corticosterone

Supplementation of antistressor package before transport significantly ($P<0.05$) affected serum corticosterone levels in the treatment groups. The serum corticosterone levels were decreased in the supplemented group having level of $133.09\mu\text{g/L}$ as compared to the non-supplemented group having levels of $154.14\mu\text{g/L}$. The different lairage environment had significant ($P<0.05$) impact on serum corticosterone levels and serum corticosterone was found less in cooler lairage temperature group having levels of $139.44\mu\text{g/L}$ as compared to normal lairage temperature group having levels of $140.68\mu\text{g/L}$ and control group having levels of $150.72\mu\text{g/L}$. The serum corticosterone levels were significantly ($P<0.05$) decreased in the interaction (between antistressor package supplementation and lairage environment). Serum corticosterone was found higher in Treatment group T_1 having levels of $172.92\mu\text{g/L}$ and lower in T_6 having levels of $125.85\mu\text{g/L}$.

4.3.2. H/L ratios

Supplementation of antistressor package before transport significantly ($P<0.05$) decreased the H/L ratio in the treatment groups. The H/L ratio was decreased in the supplemented group and was 0.49 as compared to the non-supplemented group which was 1.17. The different lairage environment also had significant ($P<0.05$) impact on the H/L ratio and the H/L ratio was found less in cooler lairage temperature group which was 0.623 as compared to normal lairage temperature group which was 0.858 and control group which was 1.004. The interaction of antistressor package supplementation and lairage environment had significant ($P<0.05$) positive impact on the H/L ratios. The H/L ratio was found higher in Treatment group T_1 which was 1.45 and lower in T_6 which was 0.39.

4.4 HSP-70 Gene expression

The relative fold expression of HSP-70 gene in jejunum sample of broiler chickens is given in Table 4.4. in which T_1 is taken as control and T_2 - T_6 was compared treatment group. The expression of HSP-70 gene was down regulated in T_4 , T_5 and T_6 treatment group. In the present study the effect of antistressor package supplementation and lairage environment on HSP-70 gene is presented in table 4.4. Supplementation of anti-stressor package before

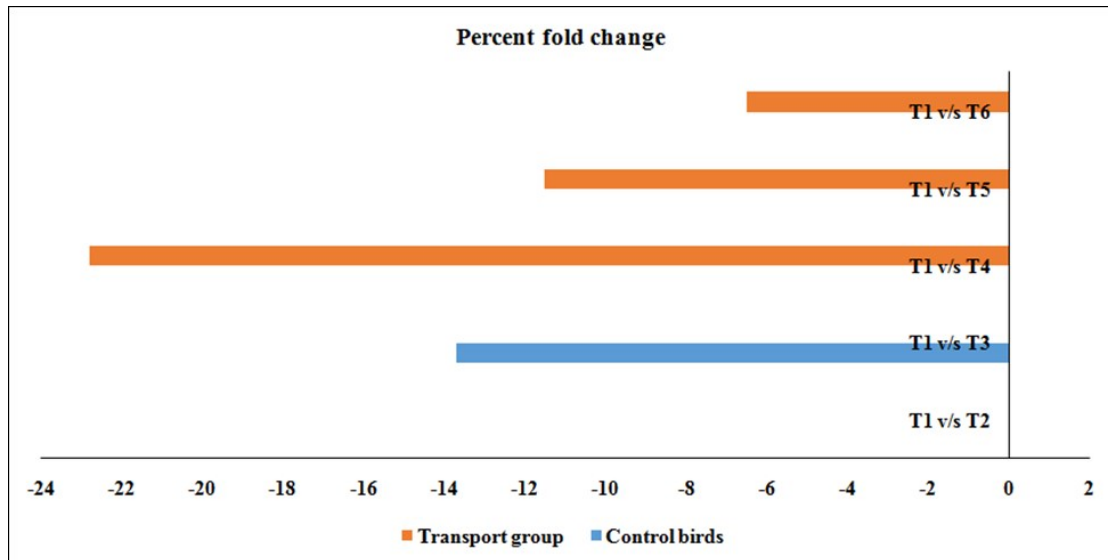
Table 4.4 Effect of antistressor package supplementation and lairage environment on differential expression of HSP 70 gene in Jejunum.

Gene	Treatments	Reaction Expression efficiency		Std. error	95 % C.L.	(HI)	Results
HSP-70	T1	1	1				
	T2	1	1.997	0.705-5.561	0.340-11.241	0.303	NS
	T3	1	0.512	0.184-1.293	0.116-3.221	0.289	NS
	T4	1	0.228	0.088-0.604	0.056-1.197	0.05	Down
	T5	1	0.155	0.036-0.609	0.010-1.115	0.039	Down
	T6	1	0.065	0.012-0.332	0.008-0.674	0.02	Down
Gene	Type	Reaction Expression efficiency		Std. error	95 % C.L.	P (HI)	Results
HSP-70	Without Antistressor	1					
	With Antistressor	1	0.19	0.043-0.787	0.043-0.787	0	Down
Gene	Type	Reaction Expression efficiency		Std. error	95 % C.L.	P (HI)	Results
HSP-70	Control	1					
	Normal environment	1	0.669	0.129-3.152	0.020-14.573	0.543	NS
	Cooler environment	1	0.383	0.065-1.983	0.010-7.282	0.158	NS

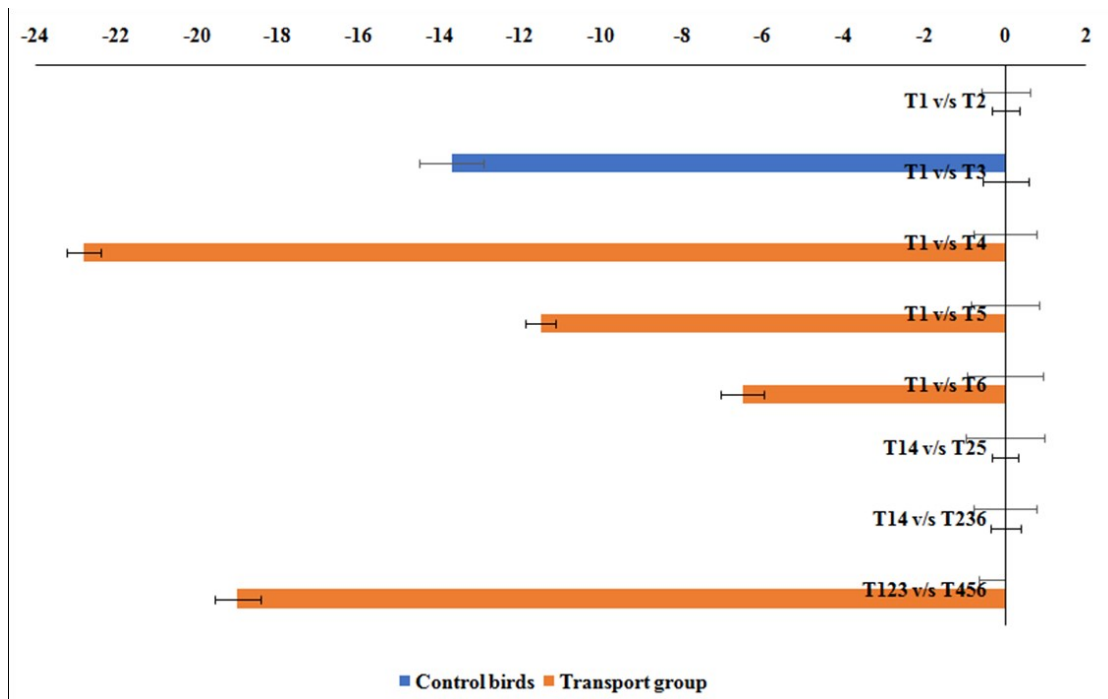
transport significantly ($P < 0.05$) down regulated the HSP-70 gene expression. Compared to control (non-supplemented) there was down regulation of HSP-70 gene in supplemented group. The expression of the HSP-70 gene was 0.19 in the supplemented group. The different lairage environments had no significant ($P > 0.05$) impact on down-regulation of HSP-70 gene expression but the expression value of HSP-70 gene was 0.383 and 0.669 in cooler and normal lairage temperature environment respectively.

4.5 Meat parameters

The result of the effect of antistressor package supplementation and lairage environments on Shear Force value, pH, drip loss, water holding capacity, and ABTS in breast muscles of broiler chickens is presented in Table 4.5.



(a)



(b)

Fig. 4.4: (a) Percent fold change on expression pattern of HSP70 gene in (Jejunum) recovered from experimental birds is indicated in bar chart. Significant down regulation of HSP70 is observed for T4, T5 and T6 group as compared to control birds ($p < 0.05$). There is no significance change observed for T2 and T7 as compared to control birds ($p < 0.05$).

(b) Percent fold change on expression pattern of HSP70 gene in (Jejunum) recovered from experimental birds is indicated in bar chart. Significant down regulation of HSP70 is observed for T4, T5 and T6 group as compared to control birds ($p < 0.05$). There is no significance change observed for T2, T7, T14 v/s T25 and T14 v/s T36 as compared to control birds ($p < 0.05$). Significant downregulation (19%) of HSP70 gene observed between T123 v/s T456 groups as compared to control birds ($p < 0.05$).

Table 4.5 Effect of antistressor package supplementation and lairage environment on meat characteristics

Treatment Grp.	pH	WHC (%)	Drip loss (%)	Shear Force (kg/cm ²)	ABTS
Anti-stressor x condition					
T1	6.0058 ^{bc} ±0.04	41.250±5.72	5.2186±0.56	0.9583±0.09	95.3750 ^a ±0.07
T2	5.8205 ^{ab} ±0.06	45.625±6.71	6.5114±0.92	0.9729±0.09	95.9286 ^b ±0.04
T3	5.9394 ^{bc} ±0.09	36.875±6.74	5.9939±0.84	0.9708±0.10	96.5000 ^c ±0.04
T4	5.6355 ^a ±0.06	40.625±2.74	4.0351±0.49	0.7833±0.08	96.7500 ^d ±0.05
T5	5.6158 ^a ±0.05	40.000±2.11	4.7739±0.76	0.6833±0.02	97.1429 ^e ±0.04
T6	6.1682 ^c ±0.07	45.625±6.22	4.9544±0.87	0.7050±0.09	97.4286 ^f ±0.04
P value	0.001	0.933	0.890	0.207	0.001
Effect of antistressor					
Without antistressor	5.922±0.04	41.250±3.61	5.908±0.45	0.967 ^b ±0.05	95.935 ^a ±0.10
With Antistressor	5.807±0.06	42.083±2.33	4.588±0.41	0.814 ^a ±0.04	97.107 ^b ±0.06
P value	0.040	0.851	0.039	0.027	0.001
Effect of lairage environment					
Control	5.821 ^a ±0.05	40.938±3.06	4.627±0.39	0.871±0.06	96.062 ^a ±0.18
Normal lairage temp	5.718 ^a ±0.05	45.625±4.42	5.643±0.62	0.828±0.06	96.536 ^b ±0.16
Cooler lairage temp	6.054 ^b ±0.06	38.437±3.44	5.474±0.60	0.973±0.06	96.964 ^c ±0.12
P value	0.001	0.407	0.368	0.204	0.001

n=10; Mean bearing different small letter (a-f) superscript row-wise differ significantly (P<0.05)

4.5.1. Shear Force Value (SFV)

Antistressor package supplementation had significant (P<0.05) impact on the SFV of the muscles of broilers in the treatment groups. Muscles of broilers in the supplemented group showed low SFV of 0.814 kg/cm² as compared to non-supplemented group of 0.967 kg/cm² whereas, different lairage environments and interaction of antistressor package supplementation and lairage environment had no significant (P>0.05) impact on SFV. SFV was less in normal lairage temperature environment group (0.828 kg/cm²) as compared to control group (0.871 kg/cm²) and cooler lairage temperature group (0.973 kg/cm²). However, under the interaction value of SF was lowest in T₅ (0.683 kg/cm²) and highest in T₂ (0.972 kg/cm²).

4.5.2. pH

Supplementation of antistressor package before transport significantly ($P < 0.05$) affected the pH of meat in the treatment groups. The pH of meat decreased in the supplemented group and was 5.807 as compared to the non-supplemented group having 5.922. The different lairage environments had significant ($P < 0.05$) impact and the pH value of meat was found less in normal lairage temperature group which was 5.71 as compared to cooler lairage temperature group and control group which was 6.05 and 5.821 respectively. Under the interaction of antistressor package supplementation and lairage condition, significant ($P < 0.05$) difference was seen in the pH of meat. The pH of meat was found higher in Treatment group T_6 which was 6.1682 and lower in T_5 which was 5.6158.

4.5.3. Drip loss

Supplementation of antistressor package before transport significantly ($P < 0.05$) affected the drip loss of the muscles of the broilers in the treatment groups. The drip loss of the muscles of the broilers was less in the supplemented group which was 4.588% as compared to the non-supplemented group which was 5.908%. Whereas, different lairage environments had no significant ($P > 0.05$) impact on drip loss%. However, drip loss% was less in control group (4.62%) as compared to cooler lairage temperature group (5.47%) and normal lairage temperature environments group (5.64%). Interaction of antistressor package supplementation and lairage environment also had no significant ($P > 0.05$) impact in all treatment groups in which T_4 (4.03%) showed the lowest drip loss percentage followed by T_5 (4.77%) and T_6 (4.95%).

4.5.4. Water holding capacity

Antistressor package supplementation, lairage environment, and interaction of antistressor package supplementation and lairage environment had no significant ($P > 0.05$) impact on the WHC value of meat. However, the values under interaction were lowest in T_3 (36.87%) and, highest in T_2 (45.62%). In different lairage environment, the WHC was less in cooler lairage temperature group (38.43%), as compared to control group (40.93%) and normal lairage temperature group (45.62%).

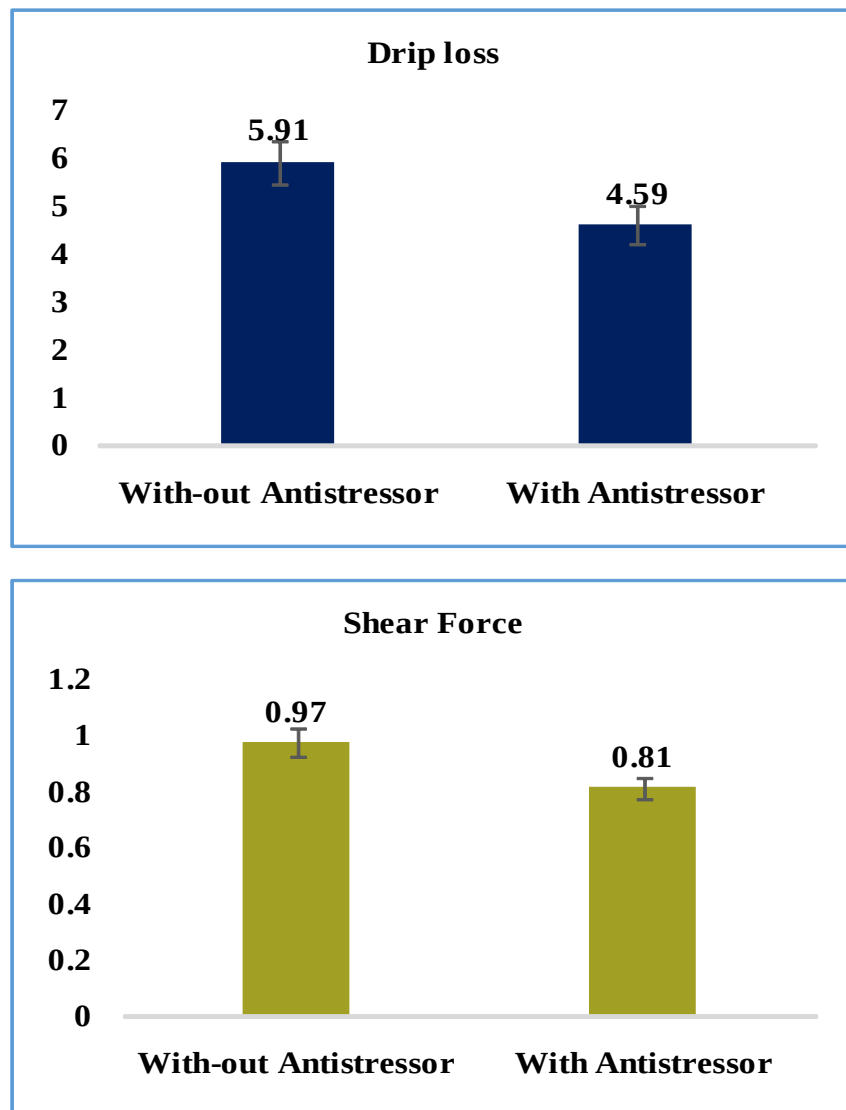


Fig. 4.5: Graphical representation of effect of antistressor package supplementation and lairage environment on meat parameters

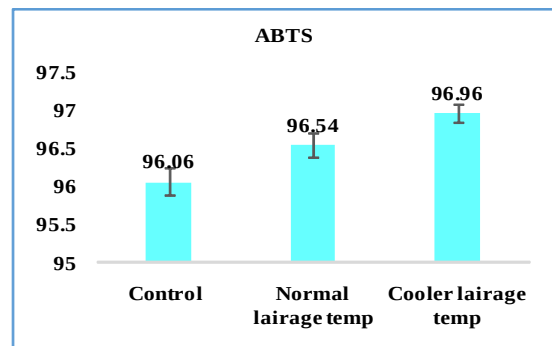
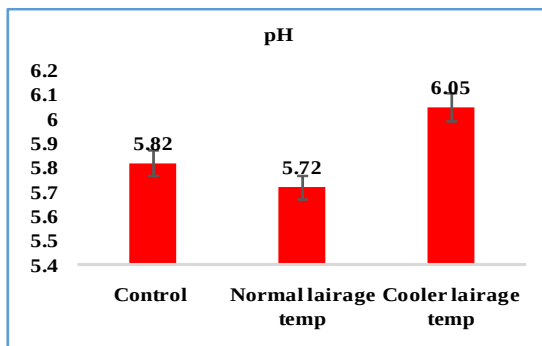
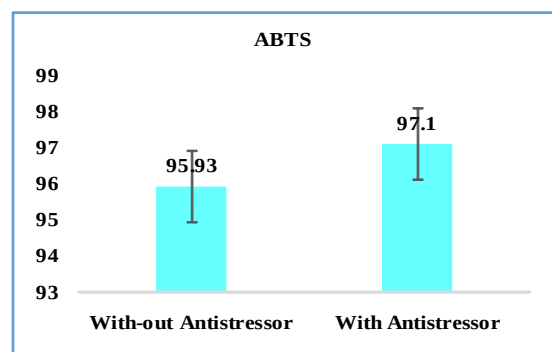
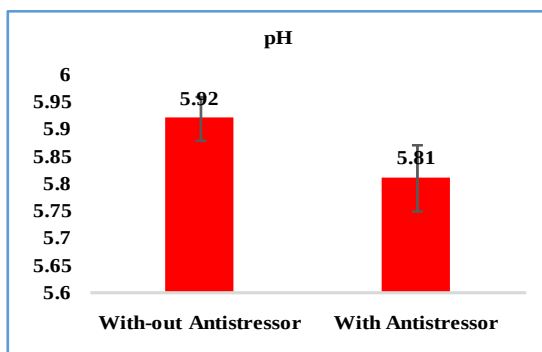
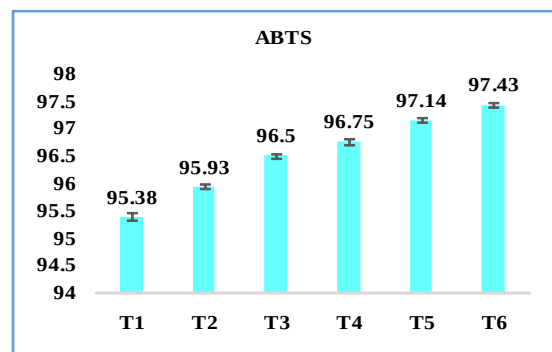
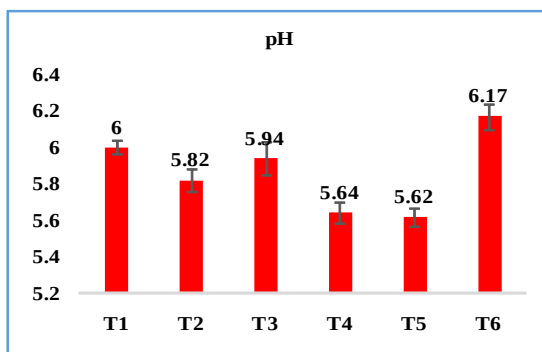


Fig. 4.5 Contd...

4.5.5. ABTS

Supplementation of antistressor package before transport significantly ($P < 0.05$) affected the ABTS value of meat in the treatment groups. The ABTS value of meat was increased in the supplemented group and was 97.107 as compared to the non-supplemented group which was 95.935. The different lairage environments also had significant ($P < 0.05$) impact on ABTS and the ABTS value of meat was found higher in cooler lairage temperature group which was 96.964 as compared to normal lairage temperature group having 96.536 and control group having 96.062. The interaction of antistressor package supplementation and lairage environment also had significant ($P < 0.05$) impact on the ABTS value of meat. The ABTS value of meat was found higher in Treatment group T_6 which was 97.42, and lower in T_1 which was 95.37.

4.6. Carcass traits

The result of effect of antistressor package supplementation and lairage environments on dressing percentage, eviscerated percentage, and cut off part yield of the carcass of broiler chickens is shown in the Table-4.6.

4.6.1. Dressing percentage

Supplementation of antistressor package before transport significantly ($P < 0.05$) affected dressing percentage in the treatment groups. The dressing percentage value was increased in the supplemented group and was 75.07% as compared to the non-supplemented group having 69.75%. The effect of lairage environment had no significant ($P > 0.05$) impact on dressing percentage. However, the dressing percentage value was significantly ($P < 0.05$) improved under interaction (between antistressor package supplementation and lairage environment). The dressing percentage of meat was found higher in Treatment group T_6 which was 76.58% and lower in T_3 which was 69.11%.

4.6.2. Eviscerated yield percentage

Supplementation of antistressor package before transport significantly ($P < 0.05$) affected eviscerated percentage in the treatment groups. The eviscerated percentage was increased in the supplemented group and was 64.44% as compared to the non-supplemented group which

Table 4.6 Effect of antistressor package supplementation and lairage environment on Carcass traits

Treatment Grp.	Dressing %	Eviscerated %	Cut-off part Yield
Anti-stressor x condition			
T1	70.4261 ^{ab} ±1.12	58.8781 ^a ±0.88	63.0653±1.44
T2	69.7164 ^a ±0.66	58.2806 ^a ±0.91	63.7874±0.71
T3	69.1154 ^a ±0.73	58.1801 ^a ±0.51	64.6960±1.02
T4	72.2723 ^a ±0.83	61.8212 ^b ±1.09	67.1031±0.47
T5	76.3696 ^c ±0.46	65.5130 ^c ±0.44	66.7190±2.24
T6	76.5861 ^c ±0.68	66.0085 ^c ±0.55	66.1672±0.86
P value	0.001	0.005	0.600
Effect of antistressor			
Without antistressor	69.753 ^a ±0.49	58.446 ^a ±0.44	63.850 ^a ±0.62
With Antistressor	75.076 ^b ±0.53	64.448 ^b ±0.54	66.663 ^b ±0.79
P value	0.001	0.001	0.009
Effect of lairage environment			
Control	71.349±0.71	60.350±0.76	65.084±0.87
Normal lairage temp	73.043±0.85	61.897±0.96	65.253±1.19
Cooler lairage temp	72.851±0.98	62.094±0.96	65.432±0.67
P value	0.065	0.055	0.963

n=10; Mean bearing different small letter (a-b) superscript row-wise differ significantly (P<0.05)

was 58.4%. The effect the of lairage environment had no significant (P>0.05) impact on eviscerated percentage. However, the interaction of antistressor package supplementation and lairage environment also had significant (P<0.05) impact on eviscerated percentage. The eviscerated percentage was found higher in Treatment group T₆ which was 66.01% and lower in T₃ which was 58.18%.

4.6.3. Cut off part yield

Antistressor package supplementation before transport significantly (P<0.05) affected cut off part yield in the treatment groups. Cut off part yield was increased in the supplemented group and was 66.66 as compared to the non-supplemented group which was 63.85. But lairage environment and interaction of antistressor package supplementation and lairage environment had no significant impact (P>0.05) on cut off part yield. However, the values

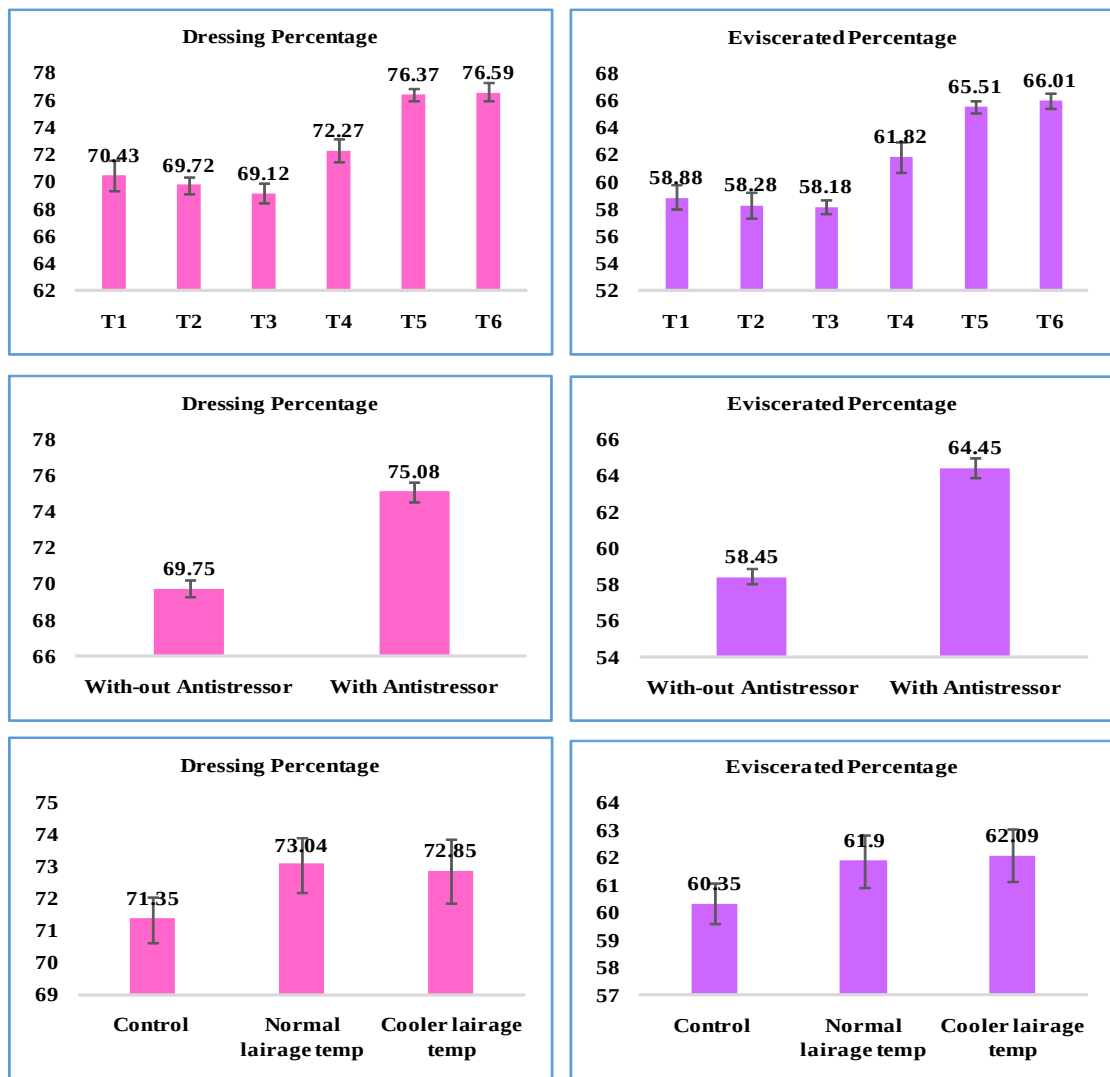


Fig. 4.6: Graphical representation of effect of antistressor package supplementation and lairage environment on carcass traits

under interaction were lowest in T₁ (63.06), and highest in T₄ (67.10). In different lairage environments, the cut off part yield was less in control group (65.08), as compared to cooler lairage temperature group (65.43) and normal lairage temperature environment group (65.25).

4.7 Serum biochemical parameters

The result of effect of antistressor package supplementation and lairage environments on serum cholesterol, uric acid, creatinine, total protein, plasma alanine and aspartate transferase levels in broiler chickens is given in Table 4.7 and 4.8.

Table 4.7 Effect of antistressor package supplementation and lairage environment on serum biochemical parameters

Treatment Grp.	Uric acid (mg/dl)	Cholesterol (mg/dl)	Total protein (g/dl)	Creatinine (mg/dl)
Anti-stressor x condition				
T1	7.1667 ^c ±0.67	220.377 ⁱ ±0.83	2.9519±0.09	1.107 ^c ±0.04
T2	6.4362 ^{bc} ±0.26	211.728 ^a ±0.57	3.3487±0.13	0.767 ^b ±0.03
T3	5.7128 ^{bc} ±0.51	206.184 ^a ±0.64	3.5311±0.10	0.696 ^b ±0.03
T4	1.5142 ^a ±0.19	198.242 ^c ±0.71	3.1784±0.08	0.642 ^b ±0.04
T5	2.8511 ^a ±0.14	180.403 ^b ±0.76	3.4609±0.09	0.464 ^a ±0.02
T6	2.0177 ^b ±0.65	166.992 ^a ±0.48	3.8677±0.20	0.339 ^a ±0.03
P value	0.001	0.001	0.658	0.038
Effect of antistressor				
Without antistressor	6.439 ^b ±0.31	212.760 ^b ±1.27	3.277 ^b ±0.08	0.857 ^b ±0.04
With Antistressor	3.128 ^a ±0.37	181.879 ^a ±2.69	3.502 ^a ±0.09	0.482 ^a ±0.03
P value	0.001	0.001	0.029	0.001
Effect of lairage environment				
Control	4.340±0.80	209.309 ^b ±2.90	3.065 ^a ±0.07	0.875 ^b ±0.06
Normal lairage temp	4.644±0.48	196.061 ^{ab} ±4.06	3.405 ^b ±0.08	0.616 ^a ±0.04
Cooler lairage temp	5.365±0.41	186.588 ^a ±5.07	3.699 ^b ±0.12	0.517 ^a ±0.05
P value	0.084	0.001	0.001	0.001

n=10; Mean bearing different small letter (a-f) superscript row-wise differ significantly (P<0.05)

4.7.1. Cholesterol and uric acid

In the present study supplementation of antistressor package before transport significantly (P<0.05) affected serum cholesterol and uric acid levels in the treatment groups.

Table 4.8 Effect of antistressor package supplementation and lairage environment on Liver Function Test

Treatment Grp.	ALT (U/L)	AST (U/L)
Anti-stressor x condition		
T1	113.25 ^f ±0.45	320.50 ^f ±0.85
T2	89.75 ^e ±0.65	285.00 ^e ±1.04
T3	85.50 ^d ±0.46	252.50 ^d ±0.87
T4	68.63 ^c ±0.60	223.25 ^c ±0.90
T5	57.50 ^b ±0.57	184.62 ^b ±1.22
T6	35.13 ^a ±0.44	143.00 ^a ±0.80
P value	0.001	0.001
Effect of antistressor		
Without antistressor	96.17 ^b ±2.56	286.00 ^b ±5.81
With Antistressor	53.75 ^a ±2.92	183.63 ^a ±6.85
P value	0.001	0.001
Effect of lairage environment		
Control	90.94 ^b ±5.77	271.88 ^b ±12.56
Normal lairage temp	73.63 ^{ab} ±4.18	234.81 ^{ab} ±12.98
Cool lairage temp	60.31 ^a ±6.51	197.75 ^a ±14.15
P value	<0.001	<0.001

n=10; Mean bearing different small letter (a-f) superscript row-wise differ significantly (P<0.05)

The serum cholesterol and uric acid levels were decreased in the supplemented group and were 181.88 & 3.13 mg/dl respectively as compared to the non-supplemented group which were 212.76 & 6.44 mg/dl respectively. The effect the of lairage environment also had significant (P<0.05) impact on serum cholesterol but not on uric acid levels and serum cholesterol level was found less in cooler lairage temperature group which was 186.58 mg/dl as compared to normal lairage temperature group which was 196.06 mg/dl and control group which was 209.31 mg/dl. Under the interaction (between antistressor package supplementation and lairage environment) the serum cholesterol and uric acid levels were significantly (P<0.05) decreased. Serum cholesterol and uric acid levels were found higher in Treatment group T₁ which were 220.37 & 7.166 mg/dl respectively and lower in T₆ which were 66.99 mg/dl and T₄ which were 1.514 mg/dl.

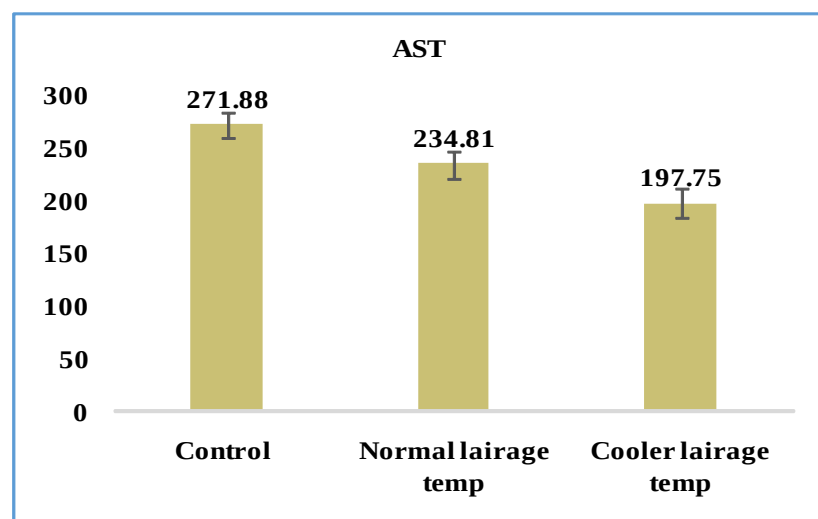
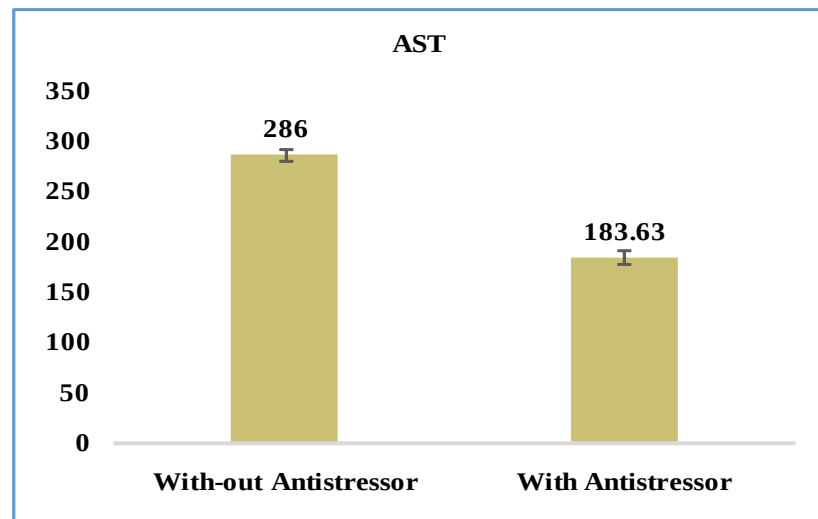
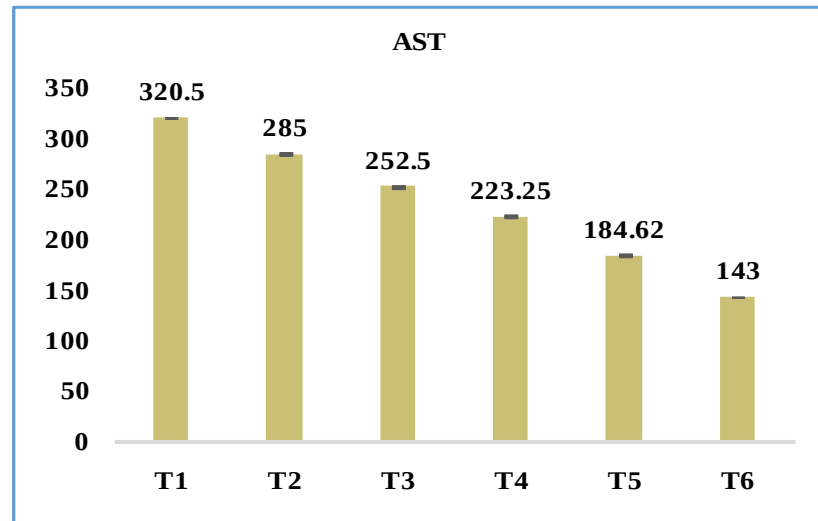


Fig. 4.7: Graphical representation of effect of antistressor package supplementation and lairage environment on serum biochemical parameters

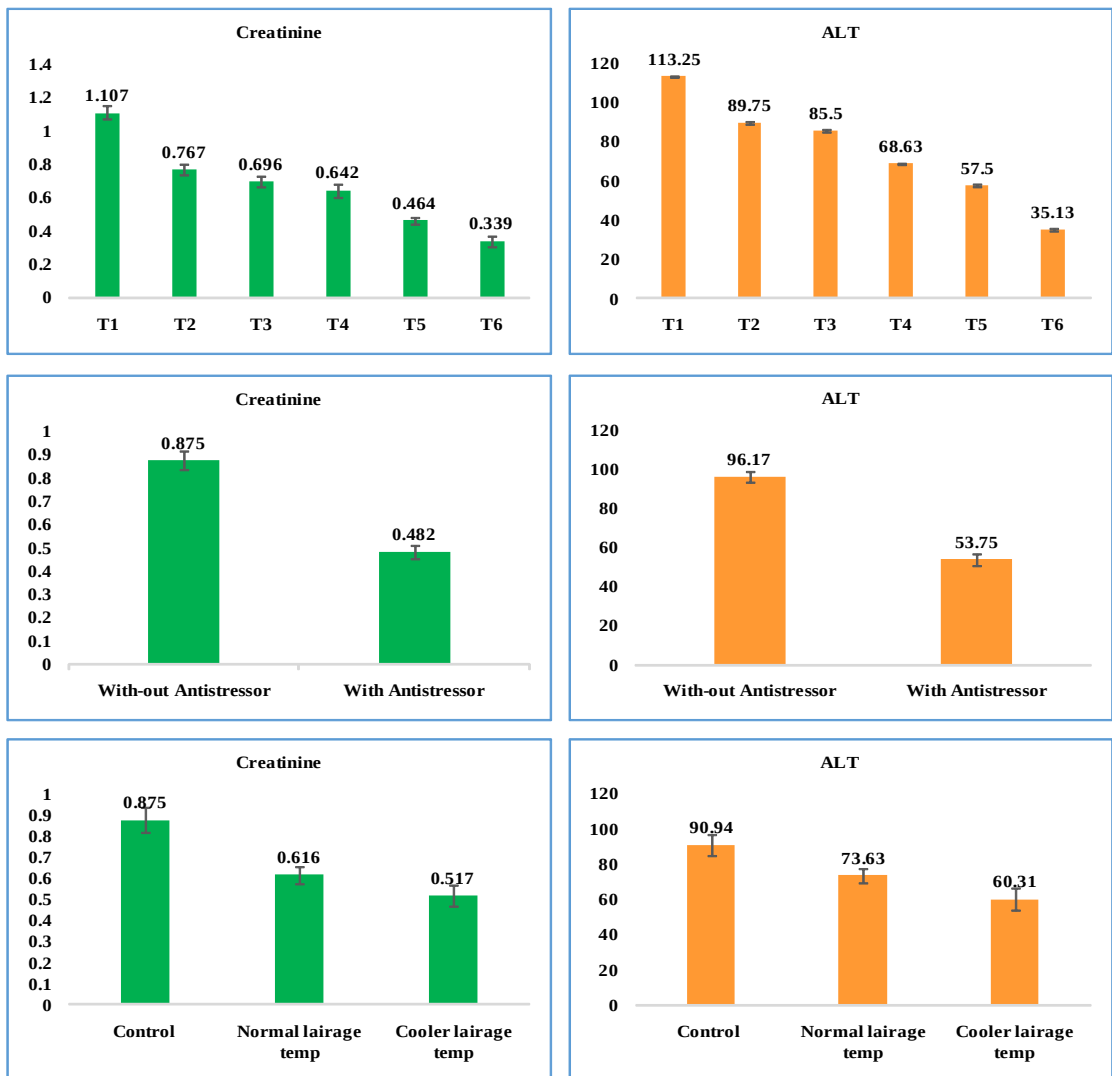


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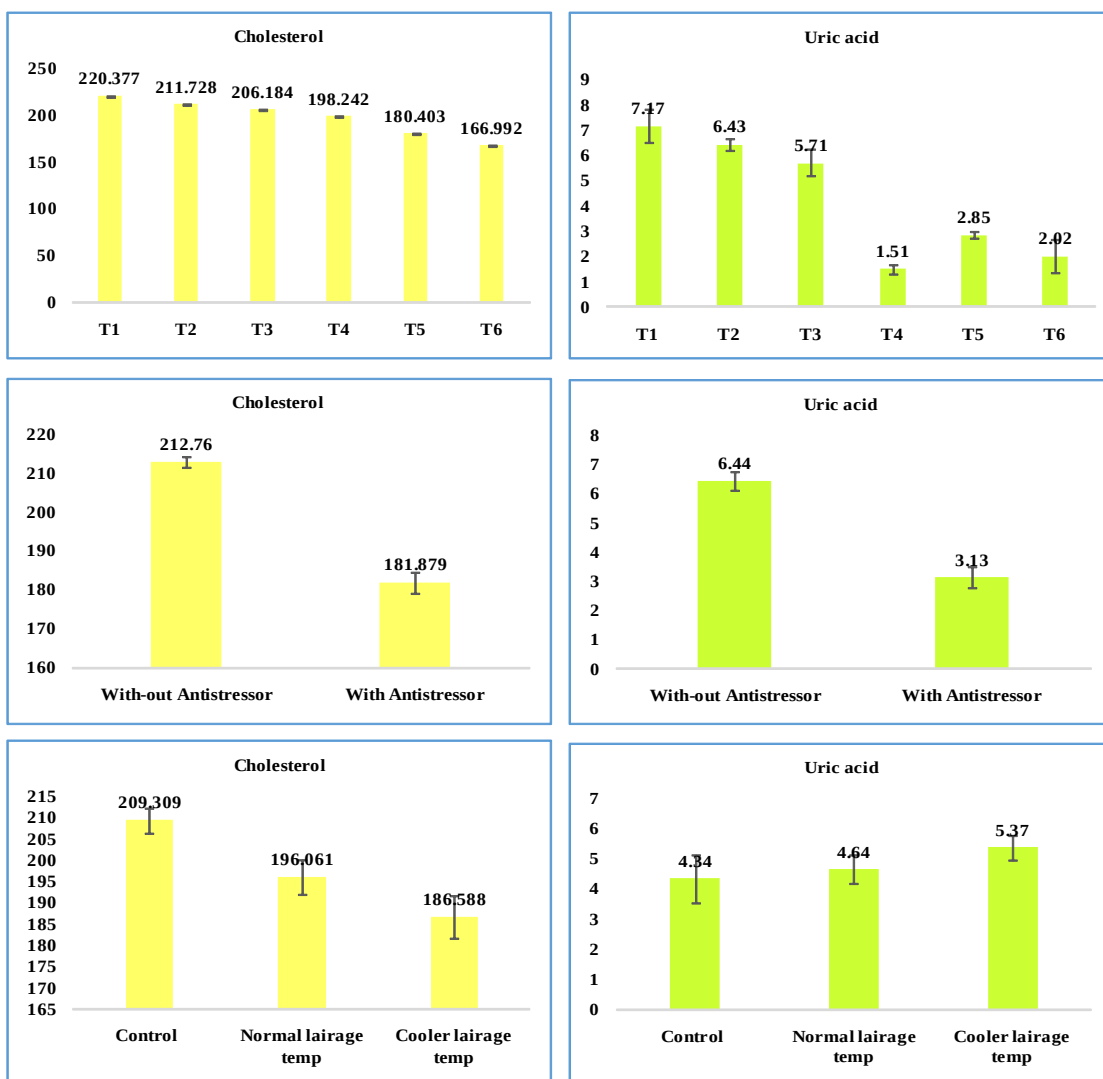


Fig. 4.7 Contd...

4.7.2. Creatinine

Supplementation of antistressor package before transport significantly ($P<0.05$) affected serum creatinine levels in the treatment groups. The serum creatinine levels were decreased in the supplemented group and were 0.482mg/dl as compared to the non-supplemented group which were 0.857mg/dl. The different lairage environment had significant ($P<0.05$) impact on serum creatinine levels and serum creatinine levels was found less in cooler lairage temperature group which was 0.517mg/dl as compared to normal lairage temperature group which was 0.616mg/dl and control group which was 0.875mg/dl. The interaction of antistressor package supplementation and lairage environment significantly ($P<0.05$) decreased the serum creatinine levels in the treatment groups. Serum creatinine level was found higher in Treatment group T_1 which was 1.107mg/dl and lower in T_6 which was 0.339mg/dl.

4.7.3. Plasma alanine and aspartate transferase (ALT & AST)

Supplementation of anti-stressor package before transport significantly ($P<0.05$) affected ALT and AST levels in the treatment groups. The ALT and AST levels were decreased in the supplemented group and were found to be 53.75 & 183.63 IU/L respectively as compared to the non-supplemented group which were 96.17 & 286 IU/L respectively. The different lairage environments had significant ($P<0.05$) impact on ALT and AST levels and ALT and AST levels were found less in the cooler lairage temperature group which was 60.31 & 197.75 IU/L respectively as compared to normal lairage temperature group which was 73.63 & 234.81 IU/L respectively and control group which was 90.94 & 271.88 IU/L respectively. Under the interaction (between antistressor package supplementation and lairage environment) the ALT and AST levels were significantly ($P<0.05$) decreased in the treatment groups. ALT and AST level was found higher in Treatment group T_1 which were 113.25 & 320.50 IU/L respectively and lower in T_6 which were 35.13 & 143 IU/L respectively.

4.7.4. Total protein

Supplementation of antistressor package before transport significantly ($P<0.05$) affected serum total protein levels in the treatment groups. The serum total protein was increased in the supplemented group and was 3.502g/dl as compared to the non-supplemented group which

was 3.277g/dl. The different lairage environment had significant ($P<0.05$) impact on total protein levels and serum total protein was found more in cooler lairage temperature group which was 3.699g/dl as compared to normal lairage temperature group which was 3.405g/dl and control group which was of 3.065g/dl. Whereas, under the interaction (between antistressor package supplementation and lairage environment) serum total protein level was not significantly improved in the treatments group. The value of total protein was highest in T_6 (3.86 g/dl) and lowest in T_1 (2.95g/dl).





Discussion

The welfare of broilers is compromised to a great extent during transportation in India because, in India, birds are mostly transported during the daytime without any shade in mostly bad road conditions. Transport stress is more when birds are travelled during the hotter part of the day without feed/water and other disturbances like noises and vibrations from the vehicle also add on in stress. This chapter discusses the results obtained in the present study which was carried out with objectives to ameliorate the transport stress in Indian conditions. The objective of the study was to find out the effect of antistressor agents, lairage environments, and their interaction on transport-induced stress, welfare, and meat quality of broilers.

5.1. Production parameters

5.1.1. Body weight change

The result of the effect of antistressor package supplementation and lairage environment on absolute and percentage change in body weight is given in Table 4.1. In the present study, significant ($P<0.05$) difference was observed in both absolute and percentage change in BW. The result of the present study was in accordance with the findings of Minka and Ayo (2010) who recorded that the percentage weight loss was less in the Ascorbic acid-supplemented group as compared to the non-supplemented group. Similarly, Attia *et al.* (2009) found significant ($P<0.05$) body weight gain in broilers when supplemented with AA and Betaine. Shanoon *et al.* (2020) also reported significant decrease in body weight loss in the salicylic acid and ascorbic acid supplemented group as compared to non-supplemented group after transport. Chen *et al.* (2020) found broilers in the betaine supplemented group showed lower weight

loss as compared to the control group. On other hand, Al-Abdullatif *et al.* (2021) found no significant change in the live weight of broilers after transport in groups which were not supplemented with different doses of betaine before transport. The effect of the lairage environment had significant impact on absolute and percentage change in body weight. The results of the present study are in line with Holm and Fletcher (1997) who reported significant decrease in body weight loss in broilers that were given a cooler (18°C) pre-slaughter environment as compared to broilers exposed to 29°C. However, no significant change was observed in the interaction group. The research findings about the interaction of the antistressor package and lairage environment were not enough, hence the result of the present study cannot be compared.

5.1.2. Mortality percentage

In the present study, no mortality was seen in treatment groups. However significant injuries were seen in non-supplemented group. The result of the present study was in accordance with the finding of Minka and Ayo (2010) who recorded 20 % mortality in the non-supplemented group and 0% in the AA-supplemented group. Similarly, Asad *et al.* (2019) recorded 2% mortality in the non-supplemented group and 0% in the supplemented group. Attia *et al.* (2009) found the highest mortality percentage in the un-supplemented group as compared to the Betaine and AA-supplemented group. Abu-Alya *et al.* (2022) also reported a significant reduction in mortality rate in the antistressor package supplemented group as compared to the non-supplemented group in long-distance transported quails. However, the data regarding the lairage environment and interaction of lairage environment and antistressor package supplementation on mortality is scarce to compare the findings of our study.

5.2. Welfare parameters

5.2.1. Gait score

Leg health is a significant problem in broilers after transportation. Transportation changes the gait pattern of commercial broilers. Results of the gait score based on the gait score index as mentioned in Webster *et al.*, (2008) are given in Table 4.2. Singh *et al.* (2013) found significant improvement in the walking ability of broilers after supplementation of aspirin. The

present study also confirms the above findings. In the present study gait score was less in the antistressor package supplemented group and this may be due to the analgesic effect of aspirin present in our anti-stressor package. Lairage environment had significant impact on gait score but to compare the effect of lairage environments on gait score limited research data was available, hence comparison of the current result is unattainable. In the current study interaction of antistressor package supplementation and lairage environment had no significant impact on gait score. There is little research available with respect to the interaction of antistressor package supplementation and lairage environment on gait score, which limits the comparison of the present study.

5.2.2. Body temperature

The results of body temperature after transport are given in Table 4.2 indicating that supplementation of anti-stressor package before transport significantly reduced the body temperature in commercial broilers. The result of the present study was in accordance with the finding of Talebi *et al.* (2011) who found that the addition of Vitamin C and glucose in drinking water decreases the effect of high temperature in broilers. Attia *et al.* (2009) also found that supplementation of AA and different doses of Betaine reduced the negative effect of heat stress and significant ($P < 0.05$) decrease in rectal temperature. Shanoon *et al.* (2020), Al-Hassani and Al-Shukri (2016) also reported a significant decrease in body temperature in the salicylic acid and ascorbic acid supplemented group as compared to the non-supplemented group after transport. The effect of the lairage environment had significant impact on body temperature. Body temperature was less in the cooler environment as compared to the normal lairage temperature and control. The result of the present study is in line with Dadgar *et al.* (2010) who found lower body temperature in the cooler environment as compared to the warmer temperature. In the current study interaction of antistressor package supplementation and lairage environment had significant impact on body temperature. There is little research available with respect to the interaction of antistressor package supplementation and lairage environment on body temperature, which limits the comparison of results obtained from the present study.

5.2.3. Runaway time

The result of the effect of antistressor package supplementation and lairage environment on runaway time is given in Table 4.2. Supplementation of antistressor package before transport significantly ($P<0.05$) affected the runaway test in the treatment groups. The value of the runaway test was decreased in the supplemented group i.e. 1.33 as compared to the non-supplemented group having 5.53. The effect of different lairage environments was found significant ($P<0.05$) on the runaway test and the runaway test was found less in the cooler lairage temperature group (2.90) as compared to the normal lairage temperature group (3.18), and the control group (4.21). The interaction of the antistressor package supplementation and lairage environment had significant ($P<0.05$) impact on the runaway test in treatment groups and treatment group T_1 recorded a maximum runaway test of 6.90 and T_6 had minimum runaway test of 1.33. There is little research available with respect to the different lairage environments and interaction of antistressor package supplementation and lairage environment on runaway test, which limits the comparison of results obtained from the present study.

5.2.4. Tonic Immobility

The results of tonic immobility (TI) are given in Table 4.2. indicated that supplementation of the antistressor package before transport decreased TI significantly ($P<0.05$) in supplemented group. The result of the present study was in accordance with Satterlee *et al.* (1994) who reported significant reduction in tonic immobility in APP supplemented group than the non-treated group. Our findings are also in agreement with the findings of Newberry and Blair (1993) and Ayo *et al.* (2014) which found a decrease in tonic immobility in broiler chickens supplemented with tryptophan and betaine respectively (antistressor). Jones *et al.* (1999) reported that supplementation of ascorbic acid reduces the tonic immobility period in stressed quails. Similarly, Egbuniwe *et al.* (2016) observed that the TI period significantly reduced in Vitamin C and betaine supplemented broilers under stress conditions. Zulkifli (2003) supplementation of ascorbic acid at the rate of 1200 ppm before transport decreased the TI period in broilers. The result of the present study was not in accordance with Perai *et al.* (2014) who did not find significant changes in tonic immobility in the Vitamin C and Chromium supplemented group and non-supplemented group. In the current study, tonic immobility was

significantly reduced in cooler environments as compared to control and normal lairage environments. But the research finding about the effect of lairage environments on tonic immobility was scanty, hence the results of the current study cannot be compared. In the current study interaction of lairage environments and antistressor package supplementation had no significant impact on TI and to compare this interaction effect, no research data was available, hence the comparison is unattainable.

5.2.5. Physical injuries

The result of the effect of antistressor package supplementation and lairage environment on physical injuries is given in Table 4.2. In the present study broilers which were supplemented with our antistressor package showed no significant difference in physical injury as compared to the non-supplemented group. The result of the present study was in accordance with Minka and Ayo (2010) who found no significant difference in the physical injuries in both supplemented and non-supplemented groups. However, lairage environments and interaction of lairage environments and antistressor package had no significant impact on injuries. To compare the effect of lairage environments and interaction results in the present study, no research data was available, hence the comparison is unattainable.

5.2.6. Faecal score

The result of the effect of antistressor package supplementation and lairage environment on the faecal score is presented in Table 4.2. In the present study, no significant improvement in faecal score was found in the antistressor package supplemented group. Only quantitative reduction is seen in the supplemented group when compared with the non-supplemented group. The result of the present study was not in line with Kettunen *et al.* (2001) and Ferket (1995) who reported that supplementation of betaine in broilers and turkey significantly improved the recovery period in case of diarrhoea. Betaine enhances the water-binding capacity of intestinal cells. In the current study, faecal score was significantly improved in cooler environments as compared to control and normal lairage environments. But the research finding about the effect of lairage environments on the faecal score was not enough, hence the results of the current study cannot be compared. In the current study interaction of lairage environments and

antistressor package supplementation had no significant impact on faecal score and to compare this interaction effect, no research data was available, hence the comparison is unattainable.

5.3. Stress parameters

5.3.1. Heterophil/Lymphocyte (H/L) ratio

The H/L ratio is the most reliable index to find the stress level in broilers. Generally, H/L ratio of >0.5 indicates stress in birds. The result of the effect of antistressor package supplementation and lairage environment on the H/L ratio is presented in Table-4.3. The present study finds that significant reduction in the value of the H/L ratio (0.49) was seen in the antistressor-supplemented group which showed stress was reduced in the supplemented group. The result of the present study was in accordance with the findings of Minka and Ayo (2010) who found a significant ($P<0.05$) reduction in the value of the H/L ratio in birds supplemented with anti-stressor agent (AA, AA+E) after transport. Our result is in line with Mohammed (2010) and Abou El-Soud *et al.* (2006) who recorded that the H/L ratios of Birds supplemented with ASA in water were significantly ($P<0.05$) less than the control group. Shanoon *et al.* (2020) reported significant decrease H/L ratio in the ascorbic acid supplemented group as compared to the salicylic acid and non-supplemented group after transport. Zulkifli (2003) supplementation of ascorbic acid at the rate of 1200 ppm before transport decreased the H/L ratio in broilers. Similarly, Abu-Alya *et al.* (2022) reported a significant reduction in the H/L ratio in the antistress-supplemented group as compared to the non-supplemented group in long-distance transported quails. Nofal *et al.* (2015) also reported that supplementation of betaine in the diets of broilers under stress conditions significantly increased the H/L ratio. The finding of Nasiroleslami *et al.* (2018) was not according to our result, who did not find significant change in the H/L ratio in betaine supplemented group under stress conditions and this may be due to different strain of birds and geographical condition. In the current study, the H/L ratio was significantly reduced in cooler environments as compared to control and normal lairage environments. But the research work about the effect of lairage environments on the H/L ratio was few, hence the results of the current study cannot be compared. In the current study interaction of lairage environments and antistressor package supplementation had significant impact on the H/L ratio and to compare this interaction effect, no research data was available,

hence the comparison is unattainable.

5.3.1. Corticosterone

The result of the effect of antistressor package supplementation and lairage environment on serum corticosterone is presented in Table 4.3. In the present study, a significant decrease in serum corticosterone level was found in the antistressor package supplemented group. The result of the present study was in accordance with Richard (1998) who reported that Vitamin C supplementation reduces the level of plasma corticosterone in birds. Similarly, Kumar *et al.* (2017) demonstrated that the supplementation of betaine and betaine plus AA reduced serum corticosterone concentration. Chen *et al.* (2020) also found significant reduction in serum corticosterone levels in the broilers supplemented with betaine when compared to the non-treated group. Abu-Alya *et al.* (2022) reported a significant reduction in corticosterone value in the antistressor package (HS) supplemented group as compared to the non-supplemented group in long-distance transported quails. But the present was not in accordance with the finding of Jones *et al.* (1999) who reported that supplementation of ascorbic acid did not decrease the corticosterone level in quail under heat stress conditions. In the present study different lairage environments and the interaction of antistressor package and lairage environments significantly decreased the serum corticosterone level. To compare the result of lairage environments and interaction effect on serum corticosterone level in the present study, no research data was available, hence the comparison is unattainable.

5.4. Gene Expression of HSP-70 gene

The HSP-70 is a key component of the broilers' adaptive response and it acts by reducing lipid peroxidation, enhancing antioxidant capacity, and enhancing the activity of digestive enzymes against stress. The expression level of the HSP-70 gene in the jejunum tissue is presented in Table 4.4. In the present study, a significant decrease in the expression of the HSP-70 gene was found in the antistressor package supplemented group. The result of the present study was in accordance with Hassan *et al.* (2020) who found that the supplementation of broilers under stress with vitamin C and acetylsalicylic acid diminished the HSP-70 level

significantly ($P < 0.05$) as compared with the non-supplemented group. The present study also supports the finding of Kumar *et al.* (2017) who demonstrated that the supplementation of betaine (800mg/kg) and ascorbic acid (300mg/kg) reduced mRNA expression of HSP-70 in the liver of ducks exposed to heat stress. Hajati *et al.* (2015) also found that supplementation of Vitamin C significantly ($P < 0.05$) reduced the expression of the HSP-70 gene in broilers under stress. In the current study, lairage environments had no significant impact on the expression of the HSP-70 gene. However, the expression level was less in cooler lairage environments as compared to the control, and to compare this finding no research data was available. Under the interaction of antistressor package and lairage environments, downregulation of the HSP-70 gene was observed in treatments 4, 5, and 6. There is no research available with respect to the interaction of antistressor package supplementation and lairage environment on the downregulation of the HSP-70 gene, which limits the comparison of the present study.

5.5. Meat characteristics

5.5.1. Shear Force Value

Tenderness is an important meat trait that increases and influences consumer acceptability. Meat tenderness is scientifically measured by the SFV. The SFV obtained from Warner-Bratzler shear device is mentioned in Table 4.5 which is not significant ($P > 0.05$) between the treatments. But antistressor package supplementation had significant impact on shear force value. The result of the present study was not in line with Al-Abdullatif *et al.* (2021) who found no significant change in SFV of broilers that were or were not supplemented with different doses of betaine before transport. In the current study, the lairage environment had no significant ($P < 0.05$) impact on SFV and which supports the finding of Dadgar *et al.* (2011) who found higher shear force value in the cooler environment as compared to the warmer temperature. Similarly, Lee *et al.* (1976) and Dadgar *et al.* (2010) did not find a significant effect of a cooler (4°C) lairage environment on breast meat tenderness in broiler chickens. But Babji *et al.* (1982) in broilers and Froning *et al.* (1978) in turkeys reported cooler environment before slaughter cause increase SFV which resulted in decreased meat tenderness. Fletcher *et al.* (1997) also found higher SFV in a cooler environment (18°C) group as compared to birds exposed to 29°C. In the current study interaction of lairage

environments and antistressor package supplementation had no significant impact on shear force value and to compare this interaction effect, no research data was available, hence the comparison is unattainable.

5.5.2. pH value

Ultimate pH is an important trait to describe keeping the quality of meat. The normal pH range of chicken meat is 5.7 to 6.1. The pH values of the meat sample obtained from the present study are presented in Table 4.5. In the present study, a significant ($P < 0.05$) decrease in the pH value of breast meat was found in the antistressor package supplemented group and was equal to normal ultimate Ph. The result of the present study was not in accordance with Chen *et al.* (2020) who found a significant increase in muscle pH in betaine supplemented group as compared to the non-supplemented group. However, in the current study, a cooler lairage environment had higher muscle pH and was in accordance with Froning *et al.* (1978), Babji *et al.* (1982), and Holm and Fletcher (1997) who reported that cooler temperature lairage environment cause a moderate increase in ultimate pH of breast meat. Similarly, Dadgar *et al.* (2010 and 2011) also found the same i.e. exposure to a cooler environment before slaughter increases the pH value of meat. Fletcher *et al.* (1997) reported a significant increase (5.83) in the pH value of breast meat in groups who were a given cooler (18°C) pre-slaughter environment as compared to broilers exposed to 29°C (5.74). But the result of the current study was not in line with the finding of Lee *et al.* (1976) who did not find significant ($P > 0.05$) effect of cooler (4°C) lairage environment on breast meat muscle glycogen level which affects the pH value in broiler chicken meat. In the current study interaction of lairage environments and antistressor package supplementation significantly decreased pH value and to compare this interaction effect, little research data was available, hence the comparison is not possible.

5.5.3. Drip loss

Drip loss is a loss of high-quality protein, water, and minerals from raw meat during storage. This loss of content affects the eating quality of meat. The drip loss percentage at 24h post-mortem of the chilled meat samples in the present study is given in Table 4.5. In the current study supplementation of antistressor package had a significant ($P < 0.05$) impact on

drip loss and was according to the finding of Chen *et al.* (2020) found a significant reduction in drip loss value in the broilers supplemented with betaine when compared to the non-supplemented group. In the present study, lairage environments had no significant ($P>0.05$) impact on drip loss. The result of the current study was not in accordance with the finding of Fletcher *et al.* (1997) who reported a significant reduction in the drip loss value of breast meat of broilers which was given a cooler (18°C) pre-slaughter environment as compared to broilers exposed to 29°C . In the current study interaction of lairage environments and antistressor package supplementation had no impact on drip loss and to compare this interaction effect, little research data was available, hence the comparison is not possible.

5.5.4. Water holding capacity

WHC is the ability of meat to retain its water during the application of external force. It is the amount of bound water related to muscle protein and has a direct effect on the shrinkage of meat during storage. The result of the effect of antistressor package supplementation and lairage environment on WHC is presented in Table 4.5. In the present study, no significant ($P>0.05$) difference in WHC was found in the antistressor package supplemented group. The result of the present study was in accordance with Babji *et al.* (1982) who reported no effects of electrolyte treatment of turkeys with respect to muscle characteristics. Similarly, Al-Abdullatif *et al.* (2021) also found no significant change in the WHC of broilers which was supplemented with different doses of betaine before transport. The current study result is in contrast with the findings of Mooney *et al.* (1998) who found that supplementation of betaine in broilers leads to higher WHC. Similarly, different lairage environments also had no significant ($P>0.05$) impact on WHC. The finding of Dadgar *et al.* (2010 and 2011) was in contrast with the present study and reported that exposure to cooler environment before slaughter increases the WBC of meat. In the present study different lairage environments and the interaction of antistressor package and lairage environments had no impact on the WHC of meat. To compare the result of lairage environments and interaction effect on WHC in the present study, no research data was available, hence the comparison cannot be done.

5.5.5. ABTS assay

The effect of anti-stressor package supplementation and lairage environment on dressing percentage is given in Table 4.5. In the present study, ABTS value was significantly ($P<0.05$) increased in the antistressor supplemented group. The result of the present study was compatible with Skøivan *et al.* (2012) who reported supplementation of Vitamin C or Selenium or a combination of both significantly improved the antioxidant property of meat. Broilers supplemented with Vitamin C or Se showed less fat content in meat as compared to the non-supplemented group. Similarly, Chen *et al.* (2020) also found that dietary supplementation of betaine increased glutathione peroxide activity in muscle and antioxidant properties of meat of transported broilers. Betaine supplementation significantly decreased the MDA content of meat. The present study was not in line with the finding of Lauridsen *et al.* (1997) who found supplementation of Vita C did not affect the oxidative stability of broiler meat. In the present study, ABTS value was significantly ($P<0.05$) increased in cooler environments as compared to normal lairage environment and control. Which showed that the antioxidant property of meat was increased in a cooler lairage environment. But to compare this finding no research data is available. Similarly, in the current study interaction effect significantly increased the ABTS value in the treatment groups but again due to a lack of data comparison of the result of the present study is not possible.

5.6. Carcass traits

5.6.1. Dressing percentage

The effect of antistressor package supplementation and lairage environment on dressing percentage is given in Table 4.6. In the present study dressing percentage was significantly ($P<0.05$) increased in the anti-stressor supplemented group. The result of the present study was compatible with Attia *et al.* (2009) who found supplementation of AA and different doses of Betaine caused a significant increase in dressing percentage. Our result was in accordance with Nofal *et al.* (2015) and El-Shinnawy (2015) who reported that supplementation of betaine in the diets of broilers under stress conditions significantly increased the dressing percentage. However, in the present study different lairage environments had no significant ($P>0.05$) impact on dressing percentage. The result of the present study was in contrast with the finding of Hale *et al.* (1980) who reported increased dressing percentage when turkeys were exposed to the

cooler pre-slaughter environment (20 °C) as compared to those exposed to 40 °C before slaughter. In the current study interaction of antistressor package and lairage environments significantly increased the dressing percentage in the treatment groups but data regarding the interaction effect on dressing percentage is scarce to compare current findings.

5.6.2. Eviscerated percentage

The effect of anti-stressor package supplementation and lairage environment on eviscerated percentage was given in Table 4.6. In the current study, the eviscerated percentage was significantly ($P < 0.05$) increased in the anti-stressor supplemented group. The result of the present study was compatible with the findings of Esteve and Mack (2000) who found supplementation of different doses of Betaine caused significant improvement in dressing percentage. Similarly, Nofal *et al.* (2015), Sakomura *et al.* (2013), and El-Shinnawy (2015) reported that supplementation of betaine in the diets of broilers under stress conditions significantly increased carcass weight. Mooney *et al.* (1998) also found that supplementation of betaine in broilers leads to a higher eviscerated percentage. The finding of Babji *et al.* (1982) was in contrast with the present study who reported no effects of electrolyte treatment of turkeys on carcass yield. Similarly, Al-Abdullatif *et al.* (2021) also found no significant ($P > 0.05$) impact of the supplementation of betaine on eviscerated percentage under transport stress. In the present study different lairage environments had no significant ($P > 0.05$) impact on eviscerated percentage. The result of the present study was not in accordance with Hale *et al.* (1980) who reported increased carcass percentage when turkeys were exposed to the cooler pre-slaughter environment (20°C) as compared to those exposed to 40 °C before slaughter. In the current study interaction of antistressor package and lairage environments significantly increased the eviscerated percentage in the treatment groups but data regarding the interaction effect on eviscerated percentage is scarce to compare current findings.

5.6.3. Cut off part yield

The effect of antistressor package supplementation and lairage environment on cut off part yield is given in Table 4.6. In the current study, cut off part yield was significantly increased in the antistressor supplemented group. The result of the present study was in contrast with the

findings of Hassan *et al.* (2005) and Sakomura *et al.* (2013) who did not find significant change in cut off part yield in broilers supplemented with betaine. Al-Abdullatif *et al.* (2021) also found no significant change in cut off part yield of broilers that was or was not supplemented with different doses of betaine before transport. Similarly, Sun *et al.* (2008) and Konca *et al.* (2008) did not find a significant difference in cut off part yield with different doses of betaine in broilers. But findings of Nofal *et al.* (2015) and El-Shinnawy (2015) supported the current study and stated that supplementation of betaine in the diets of broilers under stress conditions significantly increased the cut off part yield. In the present study different lairage environments and the interaction of antistressor package and lairage environments had no impact on the cut off part yield. To compare the result of lairage environments and interaction effect on cut off part yield in the present study, no research data was available, hence the comparison cannot be done.

5.7. Serum biochemical parameters

5.7.1. Cholesterol

The effect of anti-stressor package supplementation and lairage environment on serum cholesterol is given in Table 4.7. Cholesterol's main function is to contribute to the build-up of cell membranes, but it is also a basic substance for production of bile acids in the liver and important hormones such as corticosteroids and gonadal hormones. In the current study, serum cholesterol was significantly ($P < 0.05$) decreased in the anti-stressor supplemented group. The result of the present study was according to the finding of Samar *et al.* (2014) who found that supplementation of vitamin C reduces serum cholesterol levels in birds. Similarly, Gursu *et al.* (2004) and Asad *et al.* (2019) also found the same results in the birds supplemented with anti-stressor (Vitamin C). The finding of Hajati *et al.* (2015) was also similar to the current study who found that supplementation of Vitamin C significantly ($P < 0.05$) reduced the serum cholesterol level in broilers under stress. Similarly, Shanoon *et al.* (2020) reported significant decrease in cholesterol levels in the salicylic acid and ascorbic acid supplemented group as compared to non-supplemented group after transport. But the finding of Cinar *et al.* (2014) was in contrast with the present study and did not find significant ($P > 0.05$) change in the cholesterol level in the broilers supplemented with Vitamin C, E, and a combination of both compared to the control group. In the present study different lairage environments and the

interaction of antistressor package and lairage environments had significant impacts on the serum cholesterol level. To compare the result of lairage environments and the interaction of antistressor package and lairage environments on the serum cholesterol level in the present study, no research data was available, hence the comparison cannot be done.

5.7.2. Uric acid

The effect of anti-stressor package supplementation and lairage environment on serum uric acid is given in Table 4.7. Uric acid plays an important role in regulation of oxidative stress and increased plasma uric acid level is associated with fasting and feed restriction. In the current study, serum uric acid was significantly ($P < 0.05$) decreased in the antistressor supplemented group. The result of the present study was according to the finding of Shanoon *et al.* (2020) who reported significant decrease in serum uric acid levels in the broilers supplemented with salicylic acid and ascorbic acid as compared to non-supplemented group after transport. The finding of Nasiroleslami *et al.* (2018) and Al-Abdullatif *et al.* (2021) was in contrast with the current study who found no significant difference in uric acid levels in the serum of broilers which was or was not supplemented with different doses of betaine before transport. Similarly, Perai *et al.* (2014) did not find significant changes in the uric acid levels in the Vitamin C and Chromium supplemented group and non-supplemented group. Cinar *et al.* (2014) also did not find significant change in the uric acid level in the broilers supplemented with Vitamin C, E, and a combination of both compared to the control group. In the present study different lairage environments had no impact on the uric acid level and to compare this finding no research data is available. The interaction of antistressor package and lairage environments had significant ($P < 0.05$) impact on the serum uric acid level. To compare the result of the interaction of antistressor package and lairage environments on the serum uric acid level in the present study, no research data was available, hence the comparison cannot be done.

5.7.3. Creatinine

The effect of anti-stressor package supplementation and lairage environment on serum creatinine is given in Table 4.7. In the current study, serum creatinine was significantly ($P < 0.05$)

decreased in the anti-stressor supplemented group. The result of the present study was not according to the finding of Cinar *et al.* (2014) who found no significant change in the creatinine level in the broilers supplemented with Vitamin C, E, and a combination of both compared to the control group. In the present study different lairage environments and the interaction of antistressor package and lairage environments had significant ($P < 0.05$) impacts on the serum creatinine level. To compare the result of lairage environments and the interaction of antistressor package and lairage environments on the serum creatinine level in the present study, no research data was available, hence the comparison cannot be done.

5.7.4. Alanine aminotransferase (ALT)

The effect of anti-stressor package supplementation and lairage environment on serum alanine aminotransferase is given in Table 4.8. Alanine aminotransferase (ALT) is an enzyme found mostly in liver cells. Apart from the liver, it is also found in heart and muscle cells. The function of ALT is to convert alanine into pyruvate, for cellular energy production. In the current study, serum alanine aminotransferase was significantly ($P < 0.05$) decreased in the anti-stressor supplemented group. The result of the present study was according to the finding of Shanoon *et al.* (2020) who reported a significant decrease in serum ALT levels in the broilers supplemented with salicylic acid and ascorbic acid as compared to non-supplemented group after transport. The finding of Al-Abdullatif *et al.* (2021) was in contrast with the current finding and showed no significant change in ALT level in the serum of broilers that was or was not supplemented with different doses of betaine before transport. Similarly, Perai *et al.* (2014) also found no significant ($P > 0.05$) change in the ALT levels in the Vitamin C and Chromium supplemented group and non-supplemented group. The result of Cinar *et al.* (2014) showed no significant change in the ALT level in the broilers supplemented with Vitamin C, E, and a combination of both compared to the control group. In the present study different lairage environments and the interaction of the antistressor package and lairage environments had significant impact on the serum ALT level. To compare the result of lairage environments and the interaction of antistressor package and lairage environments on the serum ALT level in the present study, no research data was available, hence the comparison cannot be done.

5.7.5. Aspartate aminotransferase (AST)

The effect of anti-stressor package supplementation and lairage environment on serum aspartate aminotransferase is given in Table 4.8. AST catalyses a reaction between the amino acids aspartate and glutamate and is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. In the current study, serum aspartate aminotransferase was significantly ($P < 0.05$) decreased in the anti-stressor supplemented group. The result of the present study was according to the finding of Shanoon *et al.* (2020) who reported significant ($P < 0.05$) decrease in serum AST levels in the broilers supplemented with salicylic acid and ascorbic acid as compared to non-supplemented group after transport. The finding of Al-Abdullatif *et al.* (2021) was in contrast with current finding and showed no significant change in AST level in serum of broilers which was or was not supplemented with different doses of betaine before transport. Perai *et al.* (2014) also found no significant change in the AST levels in the Vitamin C and Chromium supplemented group and non-supplemented group. Cinar *et al.* (2014) also did not find significant change in the AST level in the broilers supplemented with Vitamin C, E, and a combination of both compared to the control group. In the present study different lairage environments and the interaction of anti-stressor package and lairage environments had significant ($P < 0.05$) impact on the serum AST level. To compare the result of lairage environments and the interaction of anti-stressor package and lairage environments on the serum AST level in the present study, no research data was available, hence the comparison cannot be done.

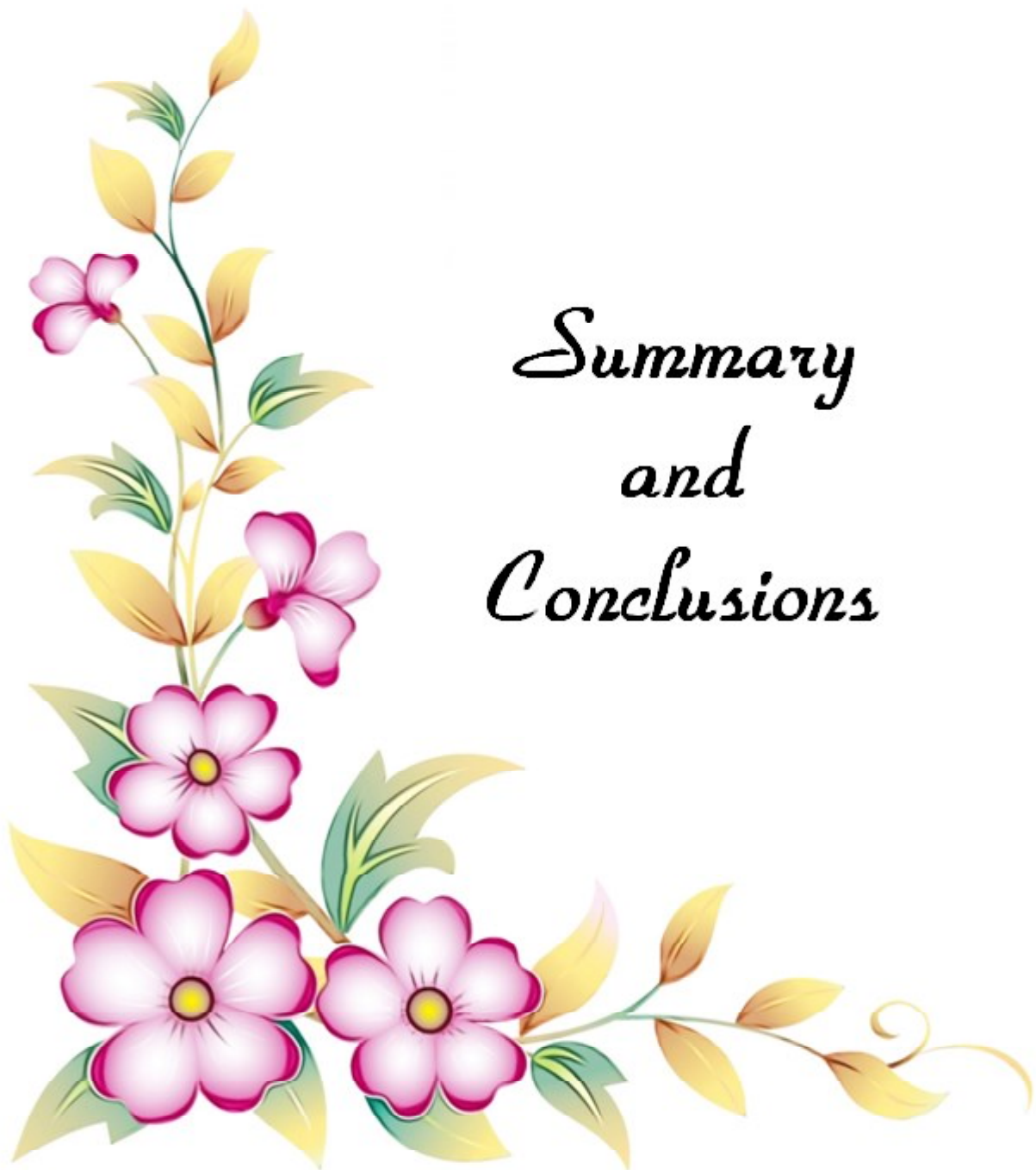
5.7.6. Total protein

The effect of anti-stressor package supplementation and lairage environment on serum total protein is given in Table 4.7. In the current study, total protein was significantly ($P < 0.05$) increased in the anti-stressor supplemented group. The result of the present study was according to the finding of Minka and Ayo (2010) who recorded the, increase in the value of total protein in both supplemented group and the control group but values were statistically significant only in the control group. The present study result contradicted the finding of Shanoon *et al.* (2020) which showed significant decrease in the value of total protein in serum in both the salicylic

acid and ascorbic acid supplemented group as compared to the non-supplemented group after transport. But Perai *et al.* (2014) did not find significant ($P>0.05$) change in total protein value in the Vitamin C and Chromium supplemented group and non-supplemented group. Similarly, Ghasemi and Nari (2020) also did not find significant difference in total protein levels in serum in betaine supplemented group under heat stress conditions. Also, the finding of Cinar *et al.* (2014) contradicted the current study and found no significant change in the total protein level in the broilers supplemented with Vitamin C, E, and a combination of both compared to the control group. In the present study different lairage environments significantly increased the total protein level and to compare this finding no research data is available. The interaction of the antistressor package and lairage environments had no significant ($P>0.05$) impact on the total protein level. To compare the result of the interaction of antistressor package and lairage environments on the total protein level in the present study, no research data was available, hence the comparison cannot be done.



*Summary
and
Conclusions*



Transportation stress is an important issue that needs to be addressed at the earliest, taken into consideration of both rearing and marketing of broiler chickens. Transportation stress is not only affecting the welfare of the broilers but also production. The economic perspective of welfare is also gaining importance nowadays, as consumers prefer food animals raised in hygienic and pristine conditions. But the conditions provided to birds during rearing as well as during transportation are totally against their wellbeing. The pathophysiology of the stresses is mainly due to the appearance of thermal discomfort, dehydration, energy depletion, and fatigue which results in weight loss. Other factors like bruises, haemorrhages lead to vascular disturbances, acid-base imbalance also electrolyte changes in their body affect birds. Not only this the catching methods during loading and unloading, vehicle condition, design of cages and ventilation system, etc. predispose chickens to more stress.

So, meat quality and welfare of broilers in the present study were primarily focused. So we have developed a product with the objective to save broilers from all types of stress, especially transportation stress. The antistressor was given to them via drinking water for 3 consecutive days before transport. Besides this, we have tried to provide different lairage environments to birds to see its effect on meat quality. The assessment of antistressor package and different lairage environments on transportation stress is done. As there was very less previous significant research work in Indian conditions in relation to broiler transport stress and its amelioration by antistressor package supplementation. The present study will be highly useful to reduce transport-induced stress and its adverse effect on commercial broilers in field conditions. Through this experiment, in near future, we will surely be able to help entrepreneurs cum small scale farmers to reduce financial losses.

- ☞ Transportation significantly ($P<0.05$) affected the percentage of body weight change and the antistressor package had a positive impact on the percentage body weight change. We have noticed percentage reduction change of 7.13% in supplemented group compared to 8.669% in non-supplemented group.
- ☞ The different lairage environment also had significant ($P<0.05$) impact on the percentage body weight change in broiler chickens and the percentage body weight change in broiler chickens was found less in cooler temperature which was -7.292% as compared to normal lairage temperature which was -7.904% and control was -8.502%. Interaction of lairage environment with antistressor had significant ($P<0.05$) impact on body weight loss.
- ☞ Significant ($P<0.05$) improvements were seen in welfare parameters like Runaway test, and gait pattern in the supplemented group, different lairage environment and interaction of lairage environment with antistressor. However, significant ($P<0.05$) impact was seen in tonic immobility only in the supplemented group. But no significant ($P>0.05$) change was observed in physical injury in all above mentioned conditions.
- ☞ Body temperature was significantly ($P<0.05$) reduced in the supplemented group which was 105.89 °F as compared to the untreated group having 108.45 °F body temperature. The body temperature was found less in cooler environment i.e. 106.53 °F as compared to normal lairage temperature having 106.91 °F and control having temperature of 108.06 °F. Under interaction condition, significant ($P<0.05$) changes occurred in body temperature.
- ☞ Heterophil/Lymphocyte ratio and corticosterone levels are the most prominent and reliable indicators of stress which always increases with increase in stress above normal level.
- ☞ H:L ratio and Corticosterone levels were significantly ($P<0.05$) reduced in the interaction, supplemented group, and in the cool lairage environment (20-25°C).
- ☞ Significant ($P<0.05$) down-regulation in the expression of the HSP-70 gene was seen in the supplemented group ($P<0.05$) only and no effect was seen in the down-regulation

of the HSP-70 gene in different lairage environments. The expression of the HSP-70 gene was down regulated in T_4 , T_5 and T_6 treatment group.

- ☞ A remarkable increase in ABTS value in all the experimented groups will lead to higher keeping quality of meat. The ABTS value of meat was increased in the supplemented group and was 97.107 as compared to the non-supplemented group which was 95.935. The pH of meat decreased in the supplemented ($P<0.05$) group and was 5.807 as compared to the non-supplemented group having 5.922.
- ☞ Drip loss and shear force value were significantly ($P<0.05$) reduced only in the treated group compared to the non-treated. However, different lairage environments and the interaction of antistressor and lairage environment did not affect the drip loss and shear force value.
- ☞ Significantly noticeable ($P<0.05$) increase in dressing %, eviscerated %, and cut-off part yield was seen only in the supplemented group as 75.07%, 64.44%, and 66.66. However different lairage environments had no significant ($P>0.05$) impact on carcass traits.
- ☞ Significant ($P<0.05$) reduction was seen in the values of LFT (AST & ALT) and KFT (Uric acid- lowest in T_4 group and creatinine).
- ☞ Except for uric acid, the lairage environment had significant ($P<0.05$) impact on cholesterol, total protein, and creatinine. Significant ($P<0.05$) reduction is seen in serum cholesterol.
- ☞ In the present study, no mortality was observed in treatment groups.
- ☞ The birds of the supplemented group did not suffer any welfare issues based on the results of welfare parameters like runway time, gait score, tonic immobility, and physical injuries. However, the H/L ratio, cholesterol level, and corticosterone levels were in the normal range in the supplemented group which may be attributed due to the capacity of our antistressor package to ameliorate the transport stress.

Conclusions

From the above study, it can be concluded that the welfare of the birds was affected significantly when transported for a period of more than 4h duration. Transportation stress significantly affected the meat condition and percentage reduction in body weight loss. Present research data shows that the supplementation of an antistressor package given at the dose rate of 4.2gm/L and cooler lairage environment of (20-25°C) reduces not only stress (corticosterone, H:L, etc.) but also improves welfare (Runaway test, gait pattern, etc.), meat quality along with percentage reduction in body weight loss in transport stressed birds.





With the objective to develop an antistressor package, we conducted a trial which helped us to progress with our concern to give desired welfare to broilers and reduce their stress, hence directly or indirectly increasing production. This trial was successful based on the already given theory that transportation of broilers for more than 4 hours leads to a considerable amount of stress in them. Not only antistressor, this study also focused on the lairage environment which has helped to bring down adverse post-transport effects in broilers. The goal of our study was to see the effect of antistressor package supplementation on production, welfare, HSP-70 gene expression, meat quality, and serum biochemical parameters. A total of 240 Cobb broiler chickens were procured from experimental broiler farm. Broiler chicks were reared in a deep litter housing system at experimental broiler farm at Central avian research institute for a total of 42-day period. They were supplemented with ad libitum feed and water. On the 42nd day, broilers were randomly divided into total of six treatment groups and they were kept off-feed for 12 hr before transport. Three days prior to transport last three treatment groups were given antistressor via drinking water. All treatment groups were transported for 8 hrs duration. Also, the remaining space in the vehicle was occupied by extra broilers to simulate the natural transport condition. Treatment T₁ was kept as control and was slaughtered without any lairage rest, while T₂ and T₃ were given 1 hr lairage rest at normal lairage temperature and cooler lairage environment respectively. In supplemented group, T₄ was kept as control and was slaughtered without any lairage rest. Treatment T₅ and T₆ were given 1 hr lairage rest at normal lairage temperature and cooler lairage environment respectively. After transport broilers were immediately unloaded and checked for welfare parameters, and after that slaughtered for collection of blood and meat samples for laboratory analysis. This study revealed that antistressor package supplementation affected the production, welfare, carcass quality, and serum biochemical parameters remarkably. Production parameters like percentage BW change significantly ($P<0.05$) improved in the supplemented group, cooler lairage environment, and in the interaction of lairage environment with antistressor. Welfare parameters like the Runaway test, and gait pattern significantly ($P<0.05$) improved in the supplemented group, different lairage environment, and Interaction of lairage environment with antistressor. Body temperature was significantly ($P<0.05$) reduced in the supplemented group. H:L ratio and Corticosterone levels were significantly ($P<0.05$) reduced in the interaction, supplemented group, and in the cool lairage environment. Significant ($P<0.05$) down-regulation in the expression of the HSP-70 gene was seen only in the supplemented group. The ABTS value of meat was increased in the supplemented group and Drip loss, shear force, and pH of meat decreased in the supplemented group. Significantly noticeable ($P<0.05$) increase in dressing %, eviscerated %, and cut-off part yield was seen only in the supplemented group however, different lairage environments had no significant impact on carcass traits. Significant ($P<0.05$) reduction was seen in the values of LFT (AST & ALT) and KFT. Except for uric acid, the lairage environment had significant ($P<0.05$) impact on cholesterol, total protein, and creatinine. Significant ($P<0.05$) reduction is seen in serum cholesterol. So we can conclude that the adverse effect due to transport stress can be overcome by giving antistressor package at the dose rate of 4.2 g/L along with cooler lairage environment. This will assure less production loss with suitable welfare to the commercial broiler chickens.



एक एंटीस्ट्रेसर पैकेज को विकसित करने के उद्देश्य से, हमने एक परीक्षण किया था। जिसका उद्देश्य न केवल ब्रॉयलर को वांछित कल्याण देना था बल्कि उनके तनाव को कम करना भी था तथा जिसके माध्यम से हम प्रत्यक्ष या अप्रत्यक्ष रूप से उत्पादन में वृद्धि कर सकें। पहले से दिए गए सिद्धांत के आधार पर एक परीक्षण किया गया जिसमें पाया गया कि ब्रॉयलर को 4 घंटे से अधिक परिवहन समय में ले जाने से उनमें काफी मात्रा में तनाव पैदा हो जाता है। इस अध्ययन में हमने एंटीस्ट्रेसर पैकेज के साथ-साथ लैरेज पर्यावरण पर भी ध्यान केंद्रित किया जिसने ब्रायलर मुर्गियों में प्रतिकूल परिवहन के बाद के तनाव के प्रभाव को कम करने में मदद की है। हमारे अध्ययन का लक्ष्य उत्पादन, कल्याण, एचएसपी-70 जीन की अभिव्यक्ति, मांस की गुणवत्ता और खून के विभिन्न मानकों पर एंटीस्ट्रेसर की पूरकता के प्रभाव को देखना था। प्रायोगिक ब्रायलर फार्म से कुल 240 कॉब ब्रायलर मुर्गियां इस प्रयोग के लिए ली गईं। कुल 42 दिनों की अवधि के लिए केंद्रीय पक्षी अनुसंधान संस्थान में प्रायोगिक ब्रायलर फार्म में बिछावन आवास प्रणाली में ब्रायलर चूजों को पाला गया था। उन्हें यथेच्छ अनुसार भोजन और पानी प्रदान किया गया। परीक्षण के 42वें दिन, ब्रॉयलर मुर्गियों को कुल छह उपचार समूहों में विभाजित किया गया और परिवहन से पहले बारह घंटों के लिए दाना तथा पानी बंद किया गया था। परिवहन से तीन दिन पहले अंतिम तीन उपचार समूहों को पीने के पानी के माध्यम से एंटीस्ट्रेसर दिया गया था। परीक्षण के 43वें दिन पर सभी उपचार समूहों को 8 घंटे की अवधि के लिए परिवहन कराया गया। साथ ही, वाहन में शेष जगह को प्राकृतिक परिवहन स्थिति का अनुकरण करने के लिए अतिरिक्त ब्रॉयलर मुर्गियों से भरा गया। उपचार टी1 को नियंत्रण के रूप में रखा गया और बिना किसी विश्राम प्रदान किये वध कर दिया गया। जबकि टी2 और टी3 को क्रमशः सामान्य कमरे के तापमान और ठंडे लेयरेज वातावरण में 1 घंटे का विश्राम दिया गया। पूरक समूह में, टी4 को नियंत्रण के रूप में रखा गया और बिना किसी विश्राम के प्रदान किये वध कर दिया गया। उपचार टी5 और टी6 को क्रमशः सामान्य कमरे के तापमान और ठंडे लेयरेज वातावरण में 1 घंटे का विश्राम दिया गया। परिवहन के बाद ब्रॉयलर मुर्गियों को तुरन्त उतार दिया गया और कल्याण मापदंडों के लिए जांच की गई, तत्पश्चात प्रयोगशाला विश्लेषण के लिए रक्त और मांस के नमूने एकत्र करने हेतु वध किया गया। इस अध्ययन से यह ज्ञात हुआ कि एंटीस्ट्रेसर सप्लीमेंट ने उत्पादन, कल्याण, शव की गुणवत्ता और खून के विभिन्न मानकों को उल्लेखनीय रूप से प्रभावित किया है। प्रतिशत शारीरिक भार जैसे उत्पादन मापक पूरक समूह, कूलर लैरेज पर्यावरण, और एंटीस्ट्रेसर के साथ विभिन्न लैरेज पर्यावरण देने से (पी<0.05) काफी सुधार हुआ है, विभिन्न लैरेज पर्यावरण, और गैट पैटर्न जैसे कल्याण मापदंडों में महत्वपूर्ण रूप से पूरक समूह में (पी<0.05) सुधार हुआ है, विभिन्न लैरेज पर्यावरण, और एंटीस्ट्रेसर के साथ लैरेज पर्यावरण की सहभागिता ने भी कल्याण मापदंडों में सुधार किया। पूरक समूह में शरीर का तापमान काफी कम (पी<0.05) था। पूरक समूह, विभिन्न विश्राम वातावरण, और पूरक के साथ-साथ वातावरण ने एचःएल अनुपात और कॉर्टिकोस्टेरोन का स्तर काफी हद तक (पी<0.05) कम किया। एचःएसःपी-70 जीन की अभिव्यक्ति में महत्वपूर्ण (पी<0.05) डाउन-रेगुलेशन केवल पूरक समूह में देखा गया। पूरक समूह के मांस में ऐ.बी.टी.एस. की मात्रा ज्यादा परन्तु ड्रिप हानि, शियर बल और मांस का पीएच कम पाया गया। महत्वपूर्ण रूप से ध्यान देने योग्य ड्रेसिंग प्रतिशत में वृद्धि, एक्सिडेशन प्रतिशत, और कट-ऑफ भाग की उपज केवल पूरक समूह में (पी<0.05) देखी गई थी, हालांकि, अलग-अलग लेयरेज वातावरणों से करकास लक्षणों पर कोई महत्वपूर्ण प्रभाव नहीं पड़ा। एलएफटी और केएफटी के मूल्यों में महत्वपूर्ण (पी<0.05) कमी देखी गई। यूरिक अम्ल को छोड़कर, लैरेज पर्यावरण का कोलेस्ट्रॉल, कुल प्रोटीन और क्रिएटिनिन पर महत्वपूर्ण (पी<0.05) प्रभाव प्राप्त हुआ। महत्वपूर्ण रूप से सीरम कोलेस्ट्रॉल में कमी (पी<0.05) देखी गई। जिसके उपरान्त हम यह निष्कर्ष निकाल सकते हैं कि परिवहन से हाने वाले तनाव के कारण होने वाले प्रतिकूल प्रभाव को 4.2 ग्राम प्रति लीटर की खुराक दर पर एंटीस्ट्रेसर पैकेज देकर तथा ठंडा लैरेज पर्यावरण प्रदान कर ब्रायलर मुर्गियों से यह तनाव दूर किया जा सकता है तथा यह भविष्य में प्रयोग होने से यह व्यावसायिक ब्रायलर मुर्गियों के लिए उपयुक्त कल्याण के साथ-साथ कम उत्पादन हानि को भी सुनिश्चित करेगा।



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