STRESS RELATED PHYSIOLOGICAL CHANGES IN CATTLE BROUGHT FOR SLAUGHTER

By

NIGIL MATHEW

THESIS

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Department of Physiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that this thesis entitled **"STRESS RELATED PHYSIOLOGICAL CHANGES IN CATTLE BROUGHT FOR SLAUGHTER"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

31/10/02

NIGIL MATHEW

Mannuthy

31-10-02

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Certified that the thesis entitled **"STRESS RELATED PHYSIOLOGICAL CHANGES IN CATTLE BROUGHT FOR SLAUGHTER"** is a record of research work done independently by **Sri. Nigil Mathew,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

31/10/02.

Dr. V. Ramnath
(Chairman, Advisory Committee)
Assistant Professor
Department of Physiology
College of Veterinary and
Animal Sciences, Mannuthy

Mannuthy
31-10-02

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Sri. Nigil Mathew,** a candidate for the degree of Master of Veterinary Science in Physiology, agree that the thesis entitled **"STRESS RELATED PHYSIOLOGICAL CHANGES IN CATTLE BROUGHT FOR SLAUGHTER"** may be submitted by Sri. Nigil Mathew in partial fulfilment of the requirement for the degree.

31/10/02.

Dr. V. Ramnath
Assistant Professor
(Chairman, Advisory Committee)
Department of Physiology
College of Veterinary and Animal Sciences, Mannuthy

31/10/2002

Dr. P.T. Philomina
Associate Professor and Head
Department of Physiology
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

31.10.02

Dr. P. Kuttinarayanan
Associate Professor
Meat Technology Unit
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

31/10/02

Dr. A. Kannan
Assistant Professor
Livestock Research Station
Thiruvazhamkunnu
(Member)

11/3/03

External Examiner
K. Narayanan.
Professor and Head
Dept of Veterinary Physiology
Veterinary College and
Res. Institute Namakkal

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Nigil Mathew

Dedicated to
My beloved Parents and Wife

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Introduction

1. INTRODUCTION

In the tropical and subtropical areas, animals are subjected to various adverse conditions stimulating a complex physiological condition "*the stress*". Stress can be defined as a non specific response of an animal in attempting to restrict or adapt to a change in the environment thereby maintaining homeostasis. Thus stress is a physiologic response of an animal to several exogenous and endogenous stimuli (stressors) that cause neuroendocrine activation. An animal is said to be in a state of "*stress*" if it is required to make some abnormal or extreme adjustments in its physiology or behaviour in order to cope with adverse aspects of its environment as quoted by Fraser and Broom (1990). An individual factor may be called a stressor if it contributes to the stressful nature of a system of husbandry.

There are two main types of reactions of an animal to stressor, viz., the alarm or emergency reaction and the general adaptation mechanism. The alarm reaction is the result of a sudden adverse stimuli which is reflected with an increased activity of the sympathetic nervous system resulting in an outpouring of catacholamines (noradrenaline and adrenaline). General adaptation mechanism is the essential stress reaction which is long lasting. Adrenocorticotropic hormone (ACTH) is released by anterior pituitary during stress bringing about the release of corticosteroids as cortisol and cortisone resulting in a relative decrease in carbohydrate metabolism, an increase in

protein metabolism and mobilization of fat depots. Stress in any form seriously affects the meat quality, thereby its marketability.

In modern animal husbandry practice, cattle are often exposed to stressful situations and among these, transportation is an acute stressor to which animals are subjected at least once during their life. Road transport is the most common means of moving cattle to slaughter houses in most countries and there should be considerable interest in improving transportation methods both from an economic and an animal welfare point of views. The transport of animals by road involves several potentially stressful conditions such as emotional factors like unfamiliar environment or social regrouping and repenning, climatic factors such as temperature, humidity or carbondioxide accumulation and pollutants and physical factors, such as noise or vibrations. The most important stressor on the moving vehicle is confinement and overloading, loss of balance while cornering, all of which greatly increase the risk of animal injury and damage to carcass and meat quality.

There are three major impacts of transport and handling stress on slaughter animals which makes this study much relevant. Firstly, transport and handling reduce the live weight and carcass yield. Secondly, transport and handling stress degrade the meat quality and finally it could be argued that transport and handling stress degrade the animal's well being. Transport and handling conditions including exposure of animals to factors like mixing with other animals, deprivation of feed and water, extreme environmental temperature, weather aberrations etc. are well known to contribute to dark

cutting condition. Such meat will be having increased toughness with negative impact on the palatability attributes (Schaefer and Jeremiah, 1992).

Assessment of stress and stress induced discomfort can be done by evaluating some haematological and biochemical parameters of blood and muscle. In Kerala, generally cattle for meat purpose are being brought by walk/trucking from neighbouring states for slaughter under stressful conditions. Only limited work has been carried out so far in evaluating the influence of transport stress on various physiological and biochemical parameters of cattle brought for slaughter. This study will be of importance in identifying certain stress producing factors and suggesting some precautionary measures to reduce stress of animals brought for slaughter.

The objective of the study is to evaluate and compare the effect of transport stress on certain physiological and biochemical parameters in cattle brought for slaughter at Meat Technology Unit, College of Veterinary and Animal Sciences, Mannuthy as well as at Corporation slaughter house, Kuriachira, Thrissur.

Review of Literature

2. REVIEW OF LITERATURE

Animals as a whole (both domestic and wild) are very sensitive to different types of handling procedures. They experience not only psychological stress during restraint, handling and exposure to strange environment, but also physical stresses like hunger, thirst, fatigue, injury or thermal extremes. Evaluation of short term stress brought by psychological or physical means can effectively be done by analysing certain physiological measures. In addition, the quality of the meat of such animals will also be affected by the stressors.

2.1 Effect of various stressors on animal health

2.1.1 Cattle

Acena *et al.* (1996) studied the hypothalamo-pituitary adrenal axial function in fighting bulls and reported that serum levels of cortisol and adrenocorticotropic hormone (ACTH) were direct markers of stress whereas the levels of serum glucose and adrenal cholesterol, white blood cell count, differential leucocyte count were considered as indirect markers of stress. Garcia Belenguer *et al.* (1996) observed the stress due to walking up in the mountain pastures during spring in cattle which resulted in an increase in white blood cell count with a reversal of lymphocytes/neutrophils ratio as a consequence of increased cortisol secretion. There was also an increased activity of serum enzymes namely creatine kinase (CK), lactate dehydrogenase (LDH), aspartate amino transferase (AST) and alanine amino transferase

(ALT). Ghuman *et al.* (1996) observed that torsion affected buffaloes showed significantly higher plasma cortisol and blood glucose level compared to normally calving buffaloes. According to them three important hormones that regulate blood glucose level (BGL) during stress were catacholamines, cortisol and glucagon. Thun *et al.* (1996) studied the influence of restraint in a crush for 120 min on non pregnant cattle and concluded that exposure to stress increased heart rate, body temperature and serum cortisol from a mean basal concentration of 3 ng/ml to 20 ng/ml.

Boissy *et al.* (1997) compared the effect of social isolation in a herd of heifers and reported that social separation induced struggling and a large increase in vocalization, heart rate and plasma cortisol concentration in all heifers and concluded that it had resulted in a severe psychological stress in cattle. Ghuman *et al.* (1997) reported that uterine torsion is a highly stressful reproductive disorder observed in buffaloes leading to release of greater concentration of glucocorticoids and catacholamines and concluded that plasma cortisol and blood glucose levels were the best indices of stress. Murata (1997) compared the effects of sera from calves receiving Burdizzo castration or intravenous ACTH injection, on bovine lymphocyte and neutrophil parameters and found that bovine lymphocytes, when incubated with sera from ACTH treated calves, suppressed the concanavalin A (Con-A) induced blastogenesis, while those from castrated calves showed a small but insignificant immunosuppression.

2.1.2 Other domesticated species

Gudev *et al.* (1995) reported that weaning of kids at the age of 45 days produced a stress which was reflected by an increase in plasma cortisol, which remained at high level upto the 5th day following weaning. They also observed a decrease in the level of plasma glucose at 16th hour after weaning which returned to the basal level by 5th day.

Becker *et al.* (1997) studied the endocrine and thermoregulatory responses of 6 months old pigs to acute thermal exposures and indicated that concentrations of cortisol increased significantly during both acute heat (34°C) and cold (10°C) exposures with response greater in heat than cold, suggesting that both extremes induced stress. Deguchi and Akuzawa (1997) observed an increase in plasma cortisol concentration one hour after grouping piglets with unfamiliar group and the same returned to normal level after 24 hours. The number of monocytes and phagocytic functions of monocytes and neutrophils were lower on the 1st and 8th day after grouping which was an indicative of stress, while the neutrophil counts remained the same throughout the experiment. Hennessy *et al.* (1997) observed that dogs when confined in a public animal shelter produced a prolonged activation of the hypothalamic pituitary adrenal axis leading to an increased plasma cortisol level in the initial days which later declined to reach normal values. Kovacs-Zomborszky *et al.* (1997) noticed that transport stress in pigs produced serious damage in cardiac and skeletal membrane as evidenced by an increase in the activities of plasma CK (980 U/l), LDH (>1600 U/l) and AST (67 U/l). Lawrence *et al.*

(1997) studied the plasma cortisol level in parturient sows and noted that cortisol concentrations increased during parturition irrespective of whether the sows were housed in crates or in pens, indicating that the parturition was a stress inducing phenomenon.

2.2 Effect of transport stress on animal health

2.2.1 Cattle

Crookshank *et al.* (1979) studied the effect of weaning and trucking of 50 calves, 12 h post weaning on body temperature and certain blood biochemical parameters such as cortisol, activities of alkaline phosphatase (ALP), CK, LDH, ALT, AST and concentrations of cholesterol, creatinine, glucose, urea nitrogen, uric acid, total protein, calcium, copper, iron, magnesium, inorganic phosphorus, potassium and zinc levels in the serum. Then they compared with 50 weaned calves untrucked and 50 calves trucked two weeks after weaning. They found that trucking led to a definite increase in the level of cortisol which returned to the normal within four to seven days, while weaning alone produced a marginal increase. Weight gains were least in the weaned group and were further decreased by trucking. Weaning resulted in marginal increase in the activity of CK, LDH, ALT and AST which further increased upon handling while transportation.

Kent and Ewbank (1983) noted that transportation of 6 months old calves for 6 h by road resulted in an increase in the level of plasma corticosteroid which remained about 127 nmol/l until immediately after

unloading (32 nmol/l) while the value of haematocrit reached maximum within half an hour of the commencement of the journey and returned to pre-transport levels within two hours. Total serum protein concentration in the transported calves rose significantly ($P<0.05$), when compared to values in non transported animals within four hours of journey. They also reported that an increased plasma glucose concentration within four hours and total leucocyte (WBC) count within 6 h of journey while there was significant ($P<0.001$) loss in body weight (8.4 ± 0.42 kg) due to journey.

Frank Blecha *et al.* (1984) reported an impaired blastogenic response of stimulated bovine lymphocytes to mitogen, in shipped calves. They justified that the elevated glucocorticoids in stressed calves were responsible to suppress *in vitro* interleukin 2 production by lymphocyte (which is necessary for proliferation) thus impairing the blastogenic response.

Tennessen *et al.* (1984) observed that transportation by road upto two hours need not be a stressful experience to bulls and steers and they found that there was a body weight loss of 2.2 per cent, rectal temperature showed an increment of 0.5°C and lower serum cortisol levels.

Kent and Ewbank (1986) compared the effect of transport on young calves aged 1 to 3 weeks old for different periods of six hours and 18 h and compared with calves starved for 18 h which acted as control and found that there were no significant changes in haematocrit and serum protein concentration suggesting that there was no significant dehydration during

journey. The plasma concentration of cortisol was elevated significantly soon after loading but a gradual increase to the maximum concentration was observed within 5 min after the journey had started. There was no major changes in the level of serum non-esterified fatty acids (NEFA), AST and CK but a significant increase in the number of neutrophils and a decrease in lymphocytes, while the total leucocyte count remained constant. The mean loss of weight was 1.95 kg and 2.95 kg respectively for 6 and 18 h of journey.

Kamimura *et al.* (1987) investigated the effect of transportation for 300 km on milk and blood components of dairy cattle and found that the milking cows suffered the greatest initial weight loss with decrease in milk yield. Total WBC count sharply increased, while eosinophilic number decreased immediately following transport, but both returned to pre-transit levels within four days. Serum levels of calcium and inorganic phosphorus decreased while NEFA, glucose, total protein, albumin, globulins and urea nitrogen increased after transport. Kenny and Tarrant (1987) suggested that confinement in a moving truck caused a substantial hyperglycemia, reflecting the activation of the sympathetic adrenal medullary system in response of transport stress in crossbred Friesian steers. They suggested that a short distance transport resulted in an increment of plasma cortisol concentration reflecting the activation of pituitary adrenal axis, indicating that motion of truck was a stressful event.

Cole *et al.* (1988) studied the effect of transport of feeder calves on various blood parameters and they found that calves which had undergone 12

and 24 h continuous journey, deprived of feed and water showed higher morbidity and mortality. They also observed a significant ($P<0.05$) linear relationship between the duration of transport and the total count of erythrocytes, leucocytes and the differential leucocyte count especially that of neutrophils and eosinophils, and the levels of serum enzymes such as ALT, hydroxy butyrate dehydrogenase and LDH, blood urea nitrogen (BUN), β-globulin, glucose and BUN to creatinine ratio. The changes in glucose concentration were related to various factors like adrenal gland activity, source of nutrients being absorbed from gastro intestinal tract, rate of lipolysis and/or glycogenolysis and rate of tissue utilization of nutrients. Mitchell *et al.* (1988) reported about the stress due to handling and transport in cattle resulting in an elevation of cortisol, triiodothyronine (T_3) and catacholamines resulting in an increment of circulating levels of glucose, lactate and lipid concentrations.

Murata (1989) studied the effect of four hours road transport on calves and found that sera collected just after transportation suppressed significantly ($P<0.05$) the lymphocyte blastogenesis using both Concanavalin-A (ConA) and phytohemagglutinin (PHA). He opined that serum contained immunosuppressive factors just after transportation which brought about the impairment of cellular immune function. Such factors were rapidly inactivated or removed from serum after unloading because they were considered to be unfavourable byproducts of metabolic disorders during transportation.

Francesco Agnes *et al.* (1990) reported that loading into a motionless transport simulator and exposure to noise produced by the simulator resulted in

similar hormonal responses such as an increase in serum cortisol and plasma epinephrine concentration suggesting that loading and noise had an important role in transport stress as observed in calves subjected for 30 min transport.

Murata and Hirose (1990) observed that lymphocyte blastogenesis in the whole blood of transported calves were significantly suppressed during and after transportation. They also reported that the calves showed a significant leucocytosis accompanied with neutrophilia while the plasma cortisol level was elevated from 7.7 to 48.3 and 38.5 ng/ml during and at zero hour after transportation, respectively.

Murata and Hirose (1991) observed that sera from calves which had been transported for 48 h covering 1400 km resulted in an impaired mitogen stimulated blastogenesis of bovine lymphocytes while sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed the appearance of 33 kilodalton (kDa) and 20 kDa proteins in most of the sera after transportation, which could be related to the immune suppression. Their studies on blood revealed that transported calves had a significant decrease in lymphocyte numbers without any significant change in total WBC and neutrophil counts.

Atkinson (1992) observed that transportation of calves had resulted in an increase in the concentration of total protein and skin fold thickness suggesting that there was dehydration of animals during the journey and it had recovered when the animals were kept in lairage. Transported animals spent more time

for resting and sleeping indicating that transport was exhaustive and lairage helped the animals to recover from the stress. Ten hours lairage resting was adequate to return all the values to the normal level. Tarrant *et al.* (1992) reported that an elevation in total WBC count, neutrophil numbers and a reduction in lymphocyte and eosinophil numbers in conditions of acute stress like road transportation for 24 h in steers. According to them adrenaline was known to mobilise white blood cells from the marginal pool into the general circulation, while eosinopenia and lymphopenia followed stress condition during which adrenocorticotropic hormone (ACTH) secretion triggered the release of glucocorticoids into the circulation.

Murata and Miyamoto (1993) observed that the sera of transported calves possessed suppressive activity on lymphocyte proliferative response to ConA. Furthermore, the hepatoglobin fraction obtained from acute phase sera exerted dose dependent suppression on lymphocyte blastogenesis. These circumstantial data suggested the possible involvement of bovine haptoglobin, in part act as an immunomodulator in the serum's suppression of lymphocyte blastogenesis in transported calves.

Mudron *et al.* (1994) studied the effect of vitamin E on leucocytic parameters and their functions in transported calves of 10 days age. Twenty mg of tocophecol acetate per kg body weight were administered orally to each of the calf, 24 h before loading and were transported by road for three hours. Blood samples were collected before and after transportation, examined for total and differential leucocyte counts, T-lymphocyte subpopulation, phagocytic

activity, leucocyte migration, serum immunoglobulin levels and plasma vitamin E and cortisol levels. Animals showed leucocytosis with netrophilia and lymphopenia after transportation.

Apple *et al.* (1995) concluded that an animal responds to an increased physical or psychological stress by releasing ACTH from anterior pituitary, glucocorticoids from adrenal cortex, epinephrine and norepinephrine from adrenal medulla and norepinephrine from sympathetic nerves, which all serve to adapt the body to stressors by affecting cardiovascular, energy producing (glucose, lactate and free fatty acids) and immune systems.

Warriss *et al.* (1995) studied the effect of transport for 5, 10 or 15 h on cattle and reported that a reduction in live weight ranged 4.6 per cent to 7 per cent for different time span. There was an increase in cortisol level, CK activity and urea content which indicated stress due to loading and transportation. The increase in total plasma protein and albumin indicated slight dehydration during transport. Increased levels of free fatty acids, beta hydroxybutyrate and urea even after the end of the journey indicated that animals normal pattern of feeding had been disrupted.

Grandin (1997) concluded that assessment of stress and discomfort should consider both behavioral and physiological measures and among them cortisol was a useful physiological parameter of short term stress such as handling or husbandry procedures like castration.

Schaefer *et al.* (1997) reported that transport and handling procedures imposed on beef cattle could be significant stress factors. Factors including off feed, water deprivation, unfamiliar noise and inclement weather during transport collectively resulted in live weight and carcass losses with degraded meat quality. According to them transport and handling also reduced the blood pH and glucose level. These changes were also accompanied by significant increase in the neutrophil: lymphocyte (N:L) ratio.

2.2.2 Other domesticated species

Coppinger *et al.* (1991) studied the effect of some stressors like repeated restraint on lambs, isolation of lambs from visual and tactile contact with other lambs and evaluated some endocrinological and immunological function. They found that repeated restraints activated the pituitary adrenal system and reduced some of the cell mediated immunological functions such as lymphocyte blastogenic response to PHA and ConA and production of interleukin 2.

Greenwood and Shutt (1992) concluded that transport of adult goats resulted in a significant increase in free cortisol levels in saliva as well as free and total cortisol levels in plasma, which suggested that determination of free cortisol in saliva was also an useful measure of stress in adult goats.

Dalin *et al.* (1993) studied the effect of transportation in gilts and indicated an increase in mean plasma cortisol level from a pre-loading value of 40 nmol/l to higher values of 70 nmol/l and 87 nmol/l respectively within 10 and 30 min after the commencement of transport. There was a corresponding

increase in plasma cortisteroid binding globulin concentration from 25 nmol/l to a level of 34 nmol/l till a pleateau was reached. The total WBC count was increased significantly (from 13.7 to 15.5 x 10^9/l), whereas, the absolute number of lymphocytes decreased (from 8.4 to 7.0 x 10^9/l), while the number of polymorphonuclear neutrophils increased significantly (from 4.3 to 7.2 x 10^9/l) during transport.

Nwe *et al.* (1994) studied the stress reaction in goats due to transportation and concluded that by 30 min of transport, plasma concentrations of cortisol and glucose sharply increased from the basal values. At the end of transport of three hours, cortisol increased 5 times than the basal value while glucose level by 4 times. The values returned to the basal levels three hours after the termination of transport. According to them the total number of white blood cells increased from the beginning of transportation with the peak value at two hours from the beginning. The trend of cortisol and haematological responses suggested that the animals were in a startle reaction with fear and anxiety to the unfamiliar treatments and environment, indicating the participation of sensory psychological pathway.

Bullova *et al.* (1995) observed that those sheep exposed for forced walking, the activity of LDH decreased from 4.02 Ukat/l before exercise to 3.57 Ukat/l, 20 min after exercise and the plasma glucose concentration increased from 4.07 mmol/l before exercise to 4.87 mmol/l, 20 min after exercise. Broom *et al.* (1996) indicated that 15 h road journey in sheep induced stress with an increased concentration in the cortisol and prolactin levels together with

a decrease in blood osmolality and hematocrit values. Gao-Deyi *et al.* (1996) noted that stress from transportation had resulted in an increase in total serum protein and globulin towards the end of transportation in pigs and these values were reduced in one to five days later, while BUN and triglyceride levels were at the highest levels on one to two days after transport.

Nwe *et al.* (1996) studied the effect of six hours transportation on various blood parameters in adult male Japanese native Tokara goats and found that plasma cortisol level was 42 ng/ml before transport, which rose immediately after the start to a peak value of 166 ng/ml within one hour. Plasma glucose level increased significantly during transport from a basal value of 67 mg/dl before the start to 165 mg/dl within 30 min and continued to reach a peak level of 264 mg/dl after the end of transportation. The number of eosinophils decreased immediately after the beginning of transportation, reached a minimum level at the end of the journey and then returned to the base value 12 h later. They concluded that at the start of transportation stress, the sympathetic nervous system was activated and then the adrenal medulla and cortex were simultaneously stimulated, followed by immediate physiological changes in the body. Smith *et al.* (1996) studied the effects of road transport stress in horses and observed that those horses under stress had an increased red blood cell count, packed cell volume (PCV), haemoglobin (Hb) concentration, plasma protein and cortisol concentrations with a decrease in the body weight immediately post-transport, indicative of slight dehydration.

Jago *et al.* (1997) studied the effect of road type and distance transported on physiology and carcass quality of red deer and found out that transport and pre-slaughter handling resulted in an increase in levels of glucose, cortisol as well as an increase in CK, AST and LDH activities and the incrementswere proportional to the distance covered.

Hall *et al.* (1998) investigated the differences in physiological responses to transportation of 94 sheep of eight genotypes representing upland and lowland types with a space allowance of 0.32 m^2/sheep for 45-90 min. Fourteen journeys were made and blood samples were taken before and after each journey, and it was observed that during journeys cortisol concentration increased significantly from 5.7 to 71.3 mmol/l and the value of packed cell volume declined significantly from 35.1 to 33.9%.

Lee *et al.* (2000a) studied the blood profile and muscle characters in pigs transported at stocking densities of 0.3, 0.4 and 0.5 m^2/pig for 1.5 h. Blood profile including the levels of cortisol, beta-endorphin, glucose and LDH activity were not significantly different among the various stocking densities while CK level for the 0.3 m^2 group was significantly ($P<0.05$) higher than the other groups. Lee *et al.* (2000b) investigated the changes in blood profile during transport and at lairage in Landrace pigs in a commercial livestock lorry for one to five hours and then holding in the lairage for three hours. Concentration of cortisol and beta endorphins were significantly higher immediately after transport than immediately after loading and pre-loading but significantly decreased after lairage retention to the level of pre-loading. There

was no difference in CK level immediately after loading and immediately after transport, but the CK level was significantly higher than those of pre-loading, while the level considerably decreased after lairage retention suggesting that pigs may suffer physical stress during loading and physiological stress during transportation, but they recovered after the lairage retention for three hours.

Kannan *et al.* (2000) studied the live weight shrinkage and stress responses in a total of 150 Spanish does that were transported in high or low density groups and held overnight before slaughter and observed that the mean live weight shrinkage was 10.2 ± 0.68 and 9.8 ± 0.68 kg in high density and low density groups, respectively. The blood samplings were done during preloading, post loading and on holding at 0, 1, 2, 3, 4 and 18 h after transportation and on analysis it was revealed that mean cortisol concentration increased at post-load sampling, peaked at zero hour after transportation and decreased thereafter. BUN value reached a maximum at 18 h while plasma concentration of glucose and CK increased at two hours after transportation. Results indicated that the stress response of goats on transportation started decreasing within three hours after transportation. The N:L ratio was higher at all time periods after transportation than prior to the journey, indicating a sustained effect of transportation stress on immune system. Stull and Rodiek (2000) observed that stressful events like transportation in horses resulted in the activation of hypothalamic – pituitary – adrenal axis leading to increased levels of plasma cortisol and the value of cortisol declined after the stress of transportation, which was probably due to cortisol's relatively short half life of

one to one and half hours. They also pointed out that the value of haematocrit and total plasma protein concentration were used as indicators of dehydration as evidenced by an incremental increase during transport and a decline to baseline levels during post-transport period.

2.3 Effect of transport stress on meat quality

Lacourt and Tarrant (1985) compared two different types of stress induction in Friesian bulls by mixing with strangers for a five hours period or by subcutaneous injections of adrenaline and reported that muscle glycogen concentration was lowered to 45 per cent of the resting value during mixing stress and to 37 per cent of the resting value following adrenaline treatment. Mixing stress caused greater loss in the level of muscle glycogen from the fast twitch fibre types while adrenaline caused a greater loss of glycogen from slow twitch fibre types indicating that pattern of muscle glycogen depletion varies with different types of stress. Loss of muscle glycogen level during transport of meat animals was due to the increased activity of cellular level of phosphorylase in glycogen degradation resulted in dark cutting condition with economic disadvantages including reduced organoleptic acceptance of the product and a shorter shelf life.

Jones *et al.* (1986) reported that transporting steers for 160 km and withholding feed for 24 h prior to slaughter increased 24 h pH from 5.64 to 5.75 compared with steers that were directly slaughtered from their holding pens which resulted in darker muscle colour.

Jones *et al.* (1988) compared the effects of transportation and fasting using three groups of Hereford cattle and observed that a live weight shrink of 7, 21 and 25 kg respectively for animals of group I which were fasted for 24 h before slaughter, animals of group II which were fasted for 24 h and then transported for 320 km (a total feed restriction period of 48 h) and group III which were fasted for 24 h, transported 320 km on two consecutive days (a total deprivation time of 72 h). It was concluded that fasting, transportation and mixing resulted in a significant loss in carcass weight and gutfill with an increased post slaughter muscle pH at 45 min and 24 h with dark muscle colour. Tarrant *et al.* (1988) assessed the response of Friesian steers to road transportation at low (200 kg/m^2), medium (300 kg/m^2) and high (600 kg/m^2) stocking densities and observed that plasma levels of cortisol and glucose significantly increased ($P<0.001$) with stocking density together with plasma CK activity ($P<0.001$) and carcass bruising ($P<0.001$). The latter two indicated the muscle damage. According to them, high stocking density influenced the standing orientation of animals resulting in loss of balance and they concluded that high stocking density caused considerable disadvantage to cattle during road transport which adversely affected animal welfare and lowered carcass quality when compared with lower and medium stocking densities.

Warriss (1990) concluded that much of the transport loss in body weight was associated with carcass components and not simply by gastrointestinal tract load. Schaefer and Jeremiah (1992) observed that transport and handling stress, particularly in the antimortem environment resulted in toughness of meat

and had a negative impact on palatability attributes. Tarrant *et al.* (1992) observed that long distance transportation of steers for 24 h resulted in an increase in ultimate pH value of meat. Eventhough the mean increase was small (0.1 to 0.2 pH units), practically all muscles with pH value above 6.0 were obtained in cattle transported long distances. They concluded that this elevation of muscle pH indicated rapid depletion of muscle glycogen reserves during long journeys which had a negative effect on meat quality, by increasing the probability of dark cutting beef.

Apple *et al.* (1995) noted that transport stress led to depletion of pre-slaughter glycogen levels, thus inhibiting post mortem lactic acid formation and accumulation and the ultimate pH exceeds 6.0, resulting in the formation of dark cutting condition. Wiklund *et al.* (1996) showed that pre-slaughter treatment such as lorry transport, helecopted herding etc. caused stress in rein deer bulls evidenced by lowest glycogen content of muscles and high pH value of meat. Degenerative lesions were observed in the skeletal muscles of these animals and developed a stress flavour after intensive pre-slaughter handling.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Animals

Female adult cattle which were culled from Livestock Research Station (LRS) - Thiruvazhamkunnu and Cattle Breeding Farm (CBF) - Thumburmuzhi of Kerala Agricultural University on account of low productivity and fertility reasons, were brought for slaughter at Meat Technology Unit (MTU), College of Veterinary and Animal Sciences, Mannuthy, and were utilized for the present study to evaluate the effect of transport stress on certain physiological parameters. Geographically, LRS - Thiruvazhamkunnu and CBF - Thumburmuzhi are two livestock farms located 80-100 km away from the Meat Technology Unit of Veterinary college campus, Mannuthy. Six animals each from LRS and CBF were selected and considered as group I. The animals were transported by trucking during late afternoon, for 2½ hours, to reach Meat Technology Unit of Veterinary college campus, Mannuthy in the month of February, 2001.

Group II animals comprising of randomly selected eight adult female cattle brought for slaughter by trucking at Corporation slaughter house, Kuriachira, Thrissur.

Group III animals comprising of eight adult female cattle brought for slaughter by walk covering 200 m from University Livestock Farm, Mannuthy to Meat Technology unit were considered as non-transported i.e., control group.

All animals selected for the study were having a minimum of 250 kg live body weight.

3.1.1 Collection of blood

From all animals of group I, 10 ml of blood sample was collected with and without anticoagulant (heparin – 20 U/ml) by jugular vein puncture before they were transported from the respective farms for slaughter. Blood collection was repeated from group I animals immediately after their arrival/unloading at Meat Technology Unit (MTU) and yet another collection was done from these animals, housed at lairage of Meat Technology Unit after giving 18 h of rest. Although these animals were provided with free access to drinking water at the lairage, they were made deprived of fodder at their respective farms, atleast 8 h before they were transported.

From animals of group II, blood samples were collected immediately after unloading from the truck and the collection was repeated after giving 18 h of rest at the lairage of Corporation Slaughter house, Thrissur.

The blood samples collected with anticoagulant were subjected for the estimation of various haematological parameters. From blood samples collected without anticoagulant, serum was separated by centrifuging at 3000 rpm for 20 min. and stored at –20°C till further analysis.

3.1.2 Body weight

Initial weight of animals before transport and final weight immediately on arrival at MTU, Mannuthy were recorded in animals of group I.

3.1.3 Transporting conditions

Environmental temperature, humidity and floor space availability during transport were taken into consideration in group I and II animals. The average air temperature recorded at 2 PM during February 2001 was 34.5°C with an average relative humidity of 67%. A floor space allowance of 1.88 m^2 was provided in the truck during the transport for group I animals to consider them thereafter as low density stocking group (LDS) whereas, only 0.95 m^2 floor space allowance was provided for group II animals, to treat them as high density stocking group (HDS).

3.1.4 Collection of meat samples

Fifty grams of skeletal muscle (semimembranosus) was collected from all animals of different groups within an hour of slaughter.

3.2 Estimation of haematological parameters

Volume of packed red blood cells (VPRC), total leucocyte (WBC) count and differential leucocyte counts (DLC) were carried out as per standard procedures (Sastri, 1998). From the differential leucocyte count, the neutrophil : lymphocyte (N:L) ratio was calculated.

3.2.1 Haemoglobin (Hb) concentration

Haemoglobin (Hb) concentration was estimated by Cyanmetheamoglobin method suggested by Van Kampen and Tijlstra (1965), using Haemo Check Kit (M/s. Agappe Diagnostics, India).

3.3 Mitogen induced lymphocyte blastogenic response: as suggested originally by Talwar (1983) and modified by Shibu et al. (2001).

Principle:

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

Reagents used:

1. RPMI 1640 (Hi-Media Laboratories Ltd., Mumbai): dried tissue culture medium was rehydrated to one litre using double distilled water (DDW) and pH adjusted to 7.2 and filtered through 0.22 µm cellulose acetate filter aseptically.

2. Bacto-Phytohaemagglutinin-M-1% (PHA-M) (Difco Laboratories, Detroit, Michigan, USA): freeze dried powder reconstituted with double distilled water (DDW).

3. Ficol-Paque solution (SISCO Research Laboratories Pvt. Ltd., Mumbai) for density gradient centrifugation.

4. Seven lakh U of penicillin (M/s Alembic Ltd., Vadodara) and 700 mg of streptomycin (M/s Sarabhai Piramal Pharmaceuticals Ltd., Vadodara) per litre of medium.

Procedure:

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

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

Seven ml of sterile RPMI-1640 medium was taken in a culture vial into which 150 µl PHA-M and 500 µl of solution containing 0.5 million of lymphocytes were added. The final volume was made upto 10 ml using sterile serum separated from animals of the study in triplicates. Incubation was carried out for 72 h at 37°C. At the end of each 24 h, lymphocyte number was determined haemocytometrically. The results were expressed as number of lymphocytes present per 500 µl at the end of each 24 h trial. The mitogenic

response of lymphocyte number was compared with the control as per the method suggested by Shibu *et al.* (2001).

3.4 Estimation of biochemical parameters

3.4.1 Blood glucose level (BGL)

The blood glucose level was estimated by glucose oxidase and peroxidase method (GOD/POD) using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai).

3.4.2 Activity of lactate dehydrogenase (LDH)- EC – 1.1.1.27

Activity of LDH was estimated by NADH coupled pyruvate reduction method using Ecoline® Kit (M/s E. Merck (India) Limited, Mumbai)

3.4.3 Activity of creatine kinase (CK) EC-2.7.3.2

Activity of CK was estimated by NAD coupled glucose-6-PO_4 oxidation method using Herichson's Kit (M/s Herichson Diagnostics, Texas).

3.4.4 Total serum protein level

The concentration of total serum protein was estimated by Biuret method using Ecoline® kit (M/s E. Merck (India) Limited, Mumbai).

3.4.5 Serum cortisol concentration

Concentration of serum cortisol was estimated using the gamma coat cortisol radioimmunoassay commercial kit (M/s Diasorin, Minnesota, U.S.A.).

Inter and intra assay coefficient of variation for the determination of cortisol concentration was done and it was found to be less than 10 per cent.

3.5 Determination of muscle quality parameters

3.5.1 pH of muscle sample

About 50 g of fresh meat (semimembranosus) was thoroughly minced with sharp blade and the glass electrode of the pH meter was immersed in it carefully without entrapping any air pocket around the bulb of electrode. The pH of the minced muscle sample at room temperature was read directly at one hour and six hours intervals after slaughter.

3.5.2 Muscle glycogen

Muscle glycogen was estimated as per the method suggested by Narasimhan (1971).

3.6 Statistical analysis

The data recorded were statistically analysed in order to compare the effect of transport within the group by students paired *t* test and between groups by students unpaired *t* test. Analysis of variance was carried out to compare the LDS and HDS groups (Snedecor and Cochran, 1989).

Results

4. RESULTS

4.1 Effect of transport on body weight of cattle

The average difference in the body weight of cattle before and after transport in low density stocking (LDS) group was found to be 5.75 ± 0.03 kg. The percentage reduction in the body weight by transport was 2.18 (table 1).

4.2 Effect of transport and a post-transport resting period of 18 h on haematological parameters

4.2.1 In LDS cattle

The mean volume of packed red blood cells (VPRC) value for LDS cattle before transport was 32.27 ± 0.91%, which increased to a significantly ($P<0.05$) higher level of 37.97 ± 1.56% at zero hour post-transport and later by taking rest for 18 h, it reduced to 31.92 ± 0.73%, which was almost in par with pre-transport value (table 2a).

The average haemoglobin (Hb) concentration for LDS cattle before transport was 12.14 ± 0.23 g%, which increased to reach a significantly higher ($P<0.05$) value of 12.95 ± 0.27 g% at zero hour post-transport and after 18 h post-transport it became 12.78 ± 0.27 g% (table 2a).

Table 1. Effect of transport on body weight of low density stocking group (LDS) cattle (n=12)

Sl. No.	Body weight in kg		Loss of body weight (kg)	Percentage reduction in body weight
	Pre-transport	'0' h post-transport		
1	265.90	259.97	5.93	2.23
2	264.66	258.82	5.84	2.21
3	260.94	255.37	5.57	2.13
4	262.18	256.52	5.66	2.16
5	261.56	255.95	5.61	2.14
6	265.28	259.40	5.88	2.22
7	264.04	258.25	5.79	2.19
8	262.80	257.10	5.70	2.17
9	262.59	256.90	5.69	2.17
10	264.25	258.44	5.81	2.20
11	263.73	257.96	5.77	2.19
12	263.11	257.38	5.73	2.18
Mean ± SE	263.42 ± 0.43	257.67 ± 0.40	5.75 ± 0.03	2.18

The total leucocyte (WBC) count for LDS cattle before transport was 9910 ± 150/µl which reached a significantly ($P<0.05$) higher value of 11539 ± 370/µl at zero hour post-transport and later became 10932 ± 325/µl after a resting period of 18 h (table 2a).

The average neutrophil: lymphocyte (N:L) ratio of LDS cattle was 0.356 ± 0.01 before journey, which reached a significantly ($P<0.05$) higher value of 0.419 ± 0.02 at zero hour post-transport. After giving 18 h of rest, the ratio reduced to 0.405 ± 0.01 (table 2a).

4.2.2 In HDS cattle

The mean VPRC and Hb concentrations for HDS cattle at zero hour post-transport were 41.50 ± 3.35% and 8.93 ± 0.40 g% respectively. After providing a resting period of 18 h post-transport, no significant changes were noticed in the above parameters (table 2b).

The total WBC count for HDS cattle at zero hour post-transport was 11417 ± 424/µl which reached a significantly ($P<0.05$) lower value of 10367 ± 110/µl after 18 h of rest (table 2b).

Table 2a. Effect of transport and a post-transport resting period of 18 h on haematological parameters in low density stocking group (LDS) cattle (n=12)

	VPRC (%)	Hb (g%)	Total WBC count/µl	N:L ratio
Pre-transport	32.27 ± 0.91[a]	12.14 ± 0.23[a]	9910 ± 150[a]	0.356 ± 0.01[a]
'0' h post-transport	37.97 ± 1.56[b]	12.95 ± 0.27[b]	11539 ± 370[b]	0.419 ± 0.02[b]
18 h post-transport	31.92 ± 0.73[ac]	12.78 ± 0.27[c]	10932 ± 325[c]	0.405 ± 0.01[c]

Mean ± SE in columns bearing different superscripts differ significantly (P<0.05)

Table 2b. Effect of transport and a post-transport resting period of 18 h on haematological parameters in high density stocking group (HDS) cattle (n=8)

	VPRC (%) (Mean ± SE)	Hb (g%) (Mean ± SE)	Total WBC count/µl (Mean ± SE)
'0' h post-transport	41.50 ± 3.35	8.93 ± 0.40	11417 ± 424
18 h post-transport	36.50 ± 3.55	8.65 ± 0.24	10367 ± 110
't' value	0.76 [NS]	0.48 [NS]	2.62*

* Significant at (P<0.05)

NS : Non significant

4.2.3 Between LDS and HDS cattle

The average Hb concentration in LDS and HDS cattle at zero hour post transport was 12.95 ± 0.27 and 8.93 ± 0.40 g% respectively, whereas the respective values at 18 h post-transport were 12.78 ± 0.27 and 8.65 ± 0.24 g%. There was a significant ($P<0.05$) difference between LDS and HDS cattle at both the periods of observations (table 2c).

No significant differences were observed between LDS and HDS cattle both at zero hour and 18 h post-transport in average VPRC and total WBC count.

4.3 Effect of transport and a post-transport resting period of 18 h on mitogen induced lymphocyte blastogenic response

The mitogen induced blastogenic responses of lymphocytes isolated from LDS cattle, pre and post-transport as well as after giving rest for a period of 18 h from LDS and HDS cattle are shown in table 3 and fig.1.

Those lymphocytes isolated from pre-transport LDS cattle responded to mitogen induced blastogenesis to an extend of 1.15 ± 0.07 x 10^6 per 500 µl after 72 h of incubation from a base value 0.5 x 10^6 per 500 µl, while blastogenic response of lymphocytes isolated from these cattle at zero hour post-transport was only upto 0.89 ± 0.08 x 10^6 per 500 µl. Lymphocytes collected from the above mentioned cattle, after 18 h post-transport showed a better response to mitogen induced blastogenesis to record 1.12 ± 0.09 x 10^6 per 500 µl.

Table 2c. **Comparison of the effect of transport at zero hour and a post transport resting period of 18 h on haematological parameters between low density stocking group (LDS) and high density stocking group (HDS) cattle**

Hours of rest	Groups	Haematological parameters (Mean ± SE)		
		VPRC(%)	Hb (g%)	Total WBC/µl
'0'	LDS (12)	37.97± 1.56	12.95± 0.27	11539± 370
'0'	HDS (8)	41.50± 3.33	8.93± 0.40	11417± 424
't ' value		1.10^{NS}	8.39*	0.20^{NS}
18	LDS (12)	31.92± 0.73	12.78± 0.27	10932± 325
18	HDS (8)	36.50± 3.55	8.65± 0.24	10367± 110
't' value		1.26^{NS}	9.93*	1.66^{NS}

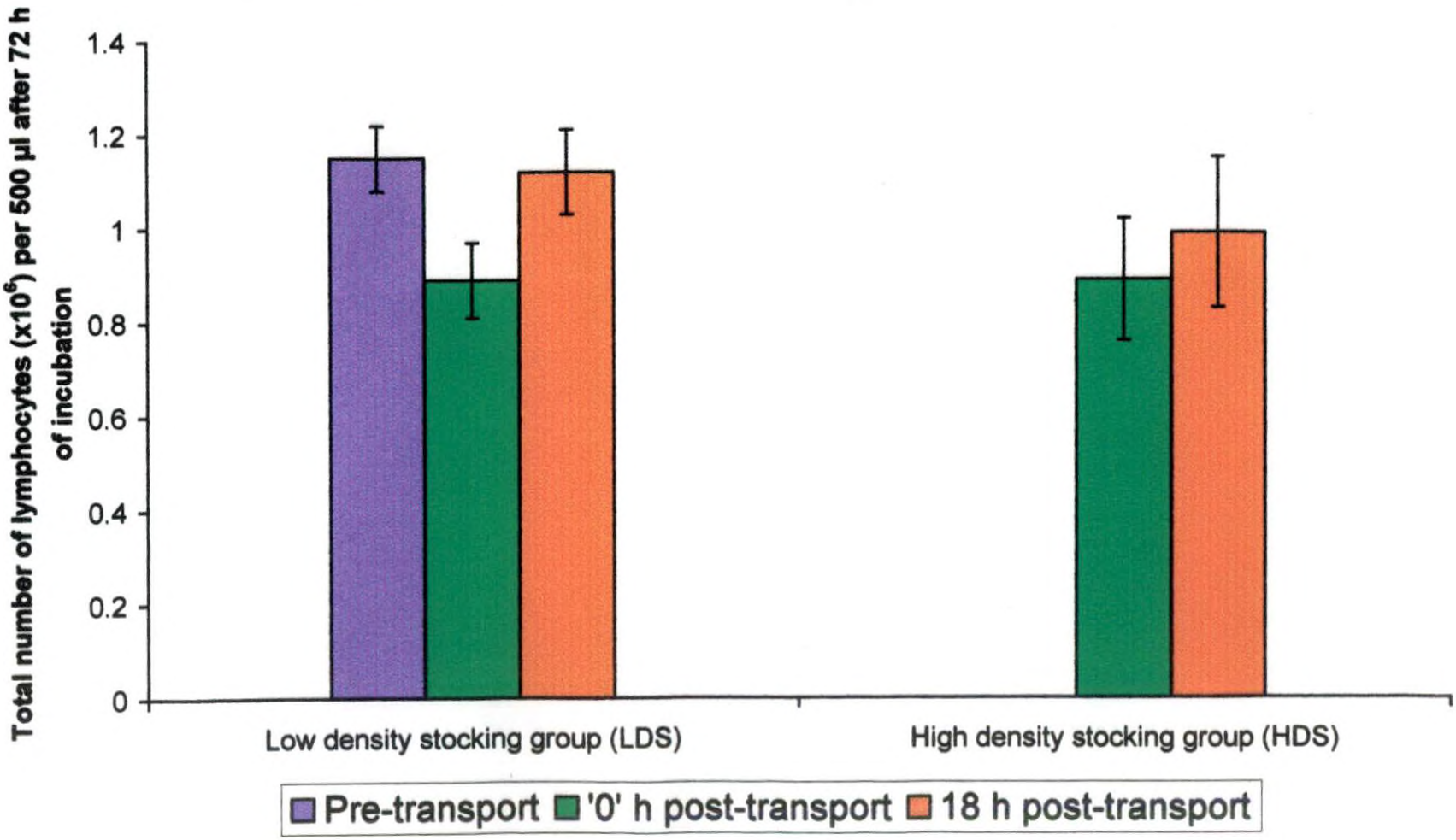
* Significant at ($P<0.05$)

NS : Non significant

Table 3. ***Mitogen induced lymphocyte blastogenic response in cattle***

Hours of rest	Number of lymphocytes ($x10^6$) per 500 µl	
	LDS group	HDS group
Pre-transport	1.15 ± 0.07	--
'0' h post-transport	0.89 ± 0.08	0.89 ± 0.13
18 h post-transport	1.12 ± 0.09	0.99 ± 0.16

Fig. I. Mitogen induced lymphocyte blastogenic response in cattle



Those lymphocytes isolated from HDS cattle at zero hour post transport responded to mitogen induced blastogenesis to an extent of 0.89 ± 0.13 x 10^6 per 500 µl after 72 h of incubation, while lymphocytes collected after giving 18 h of rest after transport showed a better response to mitogen induced blastogenesis with a value of 0.99 ± 0.16 x 10^6 per 500 µl.

4.4 Effect of transport and a post-transport resting period of 18 h on biochemical parameters

4.4.1 In LDS cattle

The average blood glucose level (BGL) for LDS cattle was 49.90 ± 2.05 mg/dl before the journey, which increased significantly ($P<0.05$) to reach 69.20 ± 4.59 mg/dl at zero hour post-transport and later on there was a non-significant reduction to a level of 62.80 ± 2.51 mg/dl after 18 h post-transport (table 4a).

The average pre-transport cortisol concentration for LDS cattle was 78.20 ± 7.48 ng/ml, which increased to a significantly ($P<0.05$) higher value of 131.20 ±18.33 ng/ml after the journey. By giving 18 h of rest the level declined significantly ($P<0.05$) to 80.50 ± 12.88 ng/ml (table 4a), which was almost in par with pre-transport value.

The average pre-transport total serum protein content in LDS cattle was 7.47 ± 0.43 g%. No significant change was observed either immediately after transport or by giving 18 h rest post-transport (table 4a).

The average pre-transport blood urea nitrogen (BUN) content of LDS cattle was 24.67 ± 0.39 mg% and it increased to reach a significantly ($P<0.05$) higher value of 27.29 ± 0.36 mg% at zero hour post-transport. After giving a period of 18 h rest, the value still increased significantly ($P<0.05$) to reach 30.36 ± 0.38 mg% (table 4a).

The average pre-transport serum creatine kinase (CK) activity in cattle of LDS group was 81.33 ± 6.54 U/l pre-transport, which later significantly ($P<0.05$) increased to 200.25 ± 12.47 U/l at zero hour post-transport. This value further increased significantly ($P<0.05$) to 235.67 ± 11.04 U/l after giving 18 h of rest (table 4a).

The average serum LDH activity in cattle of LDS group in pre-transport period was 847.58 ± 58.14 U/l. There was no significant change in the serum's LDH activity at both zero hour and 18 h post-transport periods (table 4a).

4.4.2 In HDS cattle

The average BGL observed for HDS cattle at zero hour post-transport was 87.15 ± 5.11 mg/dl which declined to a significantly ($P<0.05$) lower value of 61.77 ± 3.77 mg/dl, at 18 h post-transport (table 4b).

Other biochemical traits estimated in HDS cattle at zero hour post-transport were mean cortisol concentration, BUN and CK activity and their respective values

Table 4a. Effect of transport and a post-transport resting period of 18 h on biochemical parameters in low density stocking group (LDS) cattle (n=12)

	BGL (mg/dl	Cortisol (ng/ml)	Total serum protein (g%)	BUN (mg%)	CK (U/l)	LDH (U/l)
Pre-transport	49.90 ± 2.05[a]	78.20 ± 7.48[a]	7.47 ± 0.43[a]	24.67 ± 0.39[a]	81.33 ± 6.54[a]	847.58 ± 58.14[a]
'0' h post-transport	69.20 ± 4.59[b]	131.20 ± 18.33[b]	7.66 ± 0.36[a]	27.29 ± 0.36[b]	200.25 ± 12.47[b]	851.42 ± 42.49[a]
18 h post-transport	62.80 ± 2.51[bc]	80.50 ± 12.88[ac]	7.29 ± 0.27[a]	30.36 ± 0.38[c]	235.67 ± 11.04[c]	774.33 ± 54.98[a]

Means ± SE in columns bearing different superscripts differ significantly (P<0.05)

Table 4b. Effect of transport and a post-transport resting period of 18 h on biochemical parameters in high density stocking group (HDS) cattle (n=8)

	BGL (mg/dl) (Mean ± SE)	Cortisol (ng/ml) (Mean ± SE)	BUN (mg%) (Mean ± SE)	CK (U/l) (Mean ± SE)
'0' h post-transport	87.15 ± 5.11	160.88 ± 21.87	120.15 ± 7.73	627.34 ± 73.33
18 h post-transport	61.77 ± 3.77	114.91 ± 12.69	128.07 ± 7.12	554.28 ± 67.37
't' value	4.42*	0.91[NS]	0.29[NS]	0.72[NS]

* Significant at (P<0.05)

NS : Non significant

were 160.88 ± 21.87 ng/ml, 120.15 ± 7.73 mg% and 627.34 ± 73.33 U/l. No significant changes were observed for these parameters after giving 18 h of rest (table 4b).

4.4.3 Between LDS and HDS cattle

The mean BGL in LDS and HDS cattle at zero hour post transport were 69.20 ± 4.59 and 87.15 ± 5.11 mg/dl respectively and while comparing these two groups, they differ significantly ($P<0.05$). The BGL estimated at 18 h post transport in LDS and HDS cattle did not differ significantly (table 4c).

No significant difference was observed in the concentration of cortisol between LDS and HDS cattle, both at zero hour and 18 h post-transport.

The average BUN concentrations in LDS and HDS cattle at zero hour post-transport were 27.29 ± 0.36 and 120.15 ± 7.73 mg% respectively whereas, the respective values at 18 h post-transport were 30.36 ± 0.38 and 128.07 ± 7.12 mg%. In comparison it was found that there was significant ($P<0.05$) difference between LDS and HDS cattle at both the periods of observation (table 4c).

The mean CK activities observed in LDS and HDS cattle at zero hour post-transport were 200.25 ± 12.47 and 627.34 ± 73.33 U/l respectively, while the respective values at 18 h post-transport were 235.67 ± 11.04 and 554.28 ± 67.37 U/l. While comparing it was found that there was significant ($P<0.05$)

Table 4c. Comparison of the effect of transport at zero hour and a post-transport resting period of 18 h on biochemical parameters between low density stocking group (LDS) and high density stocking group (HDS) cattle

Hours of rest	Groups	Biochemical parameters (Mean ± SE)			
		BGL (mg/dl)	Cortisol (ng/ml)	BUN (mg%)	CK (U/l)
'0'	LDS (12)	69.20± 4.59	131.20± 18.33	27.29± 0.36	200.25± 12.47
'0'	HDS (8)	87.15± 5.11	160.88± 21.87	120.15± 7.73	627.34± 73.33
't' value		3.03*	0.60^{NS}	13.92*	2.98*
18	LDS (12)	62.80± 2.51	80.50± 12.88	30.36± 0.38	235.67± 11.04
18	HDS (8)	61.77± 3.77	114.91± 12.69	128.07± 7.12	554.28± 67.37
't' value		0.24^{NS}	1.12^{NS}	15.99*	2.42*

* Significant at ($P<0.05$)

NS : Non significant

difference between LDS and HDS cattle at both the periods of observations (table 4c).

4.5 Effect of transport stress on certain meat quality parameters

4.5.1 pH and glycogen content of meat in LDS, HDS and non transported cattle at one hour and six hours post slaughter

The muscle pH observed in LDS cattle after one hour of slaughter was 6.03 ± 0.03, which decreased significantly ($P<0.05$) to reach a pH of 5.90 ± 0.05 after six hours, while in the meat of HDS cattle there was a significant ($P<0.05$) decline in pH from 6.11 ± 0.02 at one hour to 6.01 ± 0.03 at sixth hour of slaughter. In the meat of non transported cattle, pH measured at one hour and six hour post-slaughter were 5.90 ± 0.02 and 5.72 ± 0.02 respectively, indicating a significant ($P<0.05$) decline (table 5a).

The muscle glycogen content in LDS cattle after one hour of slaughter was 1.77 ± 0.16 g% which reduced significantly ($P<0.05$) to 1.41 ± 0.16 g% after six hours, while in HDS cattle, there was a significant ($P<0.05$) decline in glycogen content from 1.43 ± 0.14 g% at one hour to 1.21 ± 0.12 g% at sixth hour of slaughter. In non-transported cattle, the muscle glycogen content was 2.42 ± 0.08 g% after one hour of slaughter which later decreased significantly ($P<0.05$) to 2.14 ± 0.06 g% after six hours (table 5a).

When the pH and glycogen content of meat samples were compared between three groups namely LDS, HDS and non-transported cattle at six hours post-slaughter, it was observed that a significant difference ($P<0.05$) in pH and glycogen content between LDS and non-transported cattle as well as between non transported and HDS cattle. While comparing the meat samples of LDS and HDS cattle significant differences could not be observed on both the pH and glycogen content at six hours post-slaughter (table 5b).

Table 5a. Comparison of pH and glycogen content of meat in low density stocking group (LDS), high density stocking group (HDS) and non-transported cattle at one hour and six hours post-slaughter

Cattle group	pH (Mean ± SE)			Glycogen (g%) (Mean ± SE)		
	1 h	6 h	't' value	1 h	6 h	't' value
LDS (12)	6.03 ± 0.03	5.90 ± 0.05	10.55*	1.77 ± 0.16	1.41 ± 0.16	8.50*
HDS (8)	6.11 ± 0.02	6.01 ± 0.03	34.30*	1.43 ± 0.14	1.21 ± 0.12	15.77*
Non-transport (8)	5.90 ± 0.02	5.72 ± 0.02	40.27*	2.42 ± 0.08	2.14 ± 0.06	15.08*

* Significant at (P<0.05)

Table 5b. Comparison of pH and glycogen content of meat samples between various groups of cattle at six hours post-slaughter

pH	Category	Mean ± SE	Mean difference	CD	Significance
After 6 h	I. LDS (12)	5.90 ± 0.05	(I & II) 0.11	0.11	NS
	II. HDS (8)	6.01 ± 0.03	(II & III) 0.29	0.12	P<0.05
	III. Non transport (8)	5.72 ± 0.02	(I & III) 0.18	0.11	P<0.05
Glycogen					
After 6 h	1. LDS (12)	1.41 ± 0.16	(I & II) 0.20	0.32	NS
	II. HDS (8)	1.21 ± 0.12	(II & III) 0.93	0.43	P<0.05
	III. Non transport (8)	2.14 ± 0.06	(I & III) 0.72	0.32	P<0.05

Values in parenthesis indicate number of animals

Discussion

5. DISCUSSION

Transportation of livestock can be dealt under 5 major aspects: (1) original environment, (2) loading, (3) journey, (4) unloading and (5) new environment. During the entire period of transport, animals experience multiplicity of stressors both physical and psychological. An assessment of physiological and biochemical stress markers will be highly useful in the evaluation of quantum of stress experienced by animals, on transport.

5.1 Effect of transport on body weight of cattle

In the present study, the percentage reduction in live weight by 2½ hours of transport (covering 80-100 km) for low density stocking (LDS) group of cattle was 2.18 per cent (table 1) which is closely agreed with reports of Tennessen *et al.* (1984) and Warriss *et al.* (1995) who reported a live weight shrinkage of 4.6 per cent after five hours and 6.5 per cent after 10 h of transport respectively. The shrinkage in body weight during transport invariably depends on various factors viz., stocking density as reported by Kannan *et al.* (2000), deprivation of feed and water, length of journey (Cole *et al.*, 1988) and local weather. Greater shrinkage in live weight was also encountered due to dehydration resulting from excessive salivation, defecation and urination, unfamiliar noise and inclement weather (Schaefer *et al.*, 1997). In the present study, the percentage shrinkage in body weight was not that much appreciable

which may be due to the ideal transporting conditions provided for LDS animals.

The average air temperature during the transport was well within the suggested lower limit of heat tolerance for ruminants (35-40°C) and comparatively lower humidity (67%) accounted for a negligible loss of body weight among LDS cattle in the present investigation.

5.2 Effect of transport and a post-transport resting period of 18 h on haematological parameters

5.2.1 In LDS cattle

Cattle in low density stocking group (LDS group) transported under ideal conditions showed some significant changes in VPRC, Hb concentration, total WBC and neutrophil counts immediately after transport and the values closely agreed with earlier observations made by Kamimura *et al.* (1987), Cole *et al.* (1988), Mudron *et al.* (1994) and Smith *et al.* (1996).

The largest source of stored erythrocytes in the body being the spleen and the spleenic contraction induced by the activity of sympathetic nerves and circulating catacholamines resulted in the changes of haematological values. It is generally considered that cortisol, catacholamines and thyroxine are the three important stress hormones (Ghuman *et al.*, 1996). In the present investigation regardless of the hydration state of the body, the adrenergic response of spleenic contraction resulted in an increased number of circulating erythrocytes with high VPRC value. As the number of erythrocytes thrown into the

circulation increased during transport stress, there was a rise in the concentration of Hb of LDS cattle at zero hour post-transport.

In the present study, LDS cattle showed a significant increase in total WBC count with neutrophilia and marginal lymphopenia. Adrenaline is known to have the capacity to mobilize leucoytes from marginal pool to the general circulation (Tarrant *et al.*, 1992) and this may be the reason for an increased total WBC count.

A significant increase in neutrophil: lymphocyte ratio was observed in LDS cattle at zero hour post-transport which was chiefly due to an absolute increase in the count of neutrophils accompanied by a marked lymphopenia as observed earlier by Cole *et al.* (1988). An increased neutrophil:lymphocyte ratio is considered as one of the useful measure of stress because only sustained effect of the stressor could induce a change like this as suggested by Kannan *et al.* (2000).

In the present investigation, most of the above mentioned haematological indices returned to the values of pre-transport level after 18 h of rest which indicated that facilities provided at the lairage for the transported cattle of LDS group was optimum that facilitated an early recovery of altered haematological traits. The important managemental conditions to be provided at the lairage are (1) free access to drinking water (2) a well ventilated shed with enough floor space for resting and (3) little greens. These could definitely

reduce the stress on cattle eventhough the lairage shed being considered as a new environment for them.

5.2.2 In HDS cattle

In the present study on high density stocking (HDS) group of cattle it was found that except for total WBC count, all the other parameters screened such as VPRC and Hb concentration did not change significantly after giving a post-transport resting period of 18 h when compared to their respective values at zero hour.

5.2.3 Between LDS and HDS cattle

While comparing the effect of transport between LDS and HDS cattle, it could be noted that HDS group showed significant variations in Hb concentration at zero hour post-transport. While comparing various haematological parameters between LDS and HDS cattle at 18 h post-transport it was found that only the value of Hb concentration varied significantly between the two groups.

5.3 Effect of transport and a post-transport resting period of 18 h on mitogen induced lymphocyte blastogenic response

In the present study *in vitro* blastogenic response of isolated lymphocytes to mitogen, indicated that those autologous sera which contained more than 80 ng/ml of cortisol resulted in a suppressed lymphocyte blastogenic response. This finding closely agreed with earlier observations made by Frank

Blecha *et al.* (1984), Murata (1989), Murata and Hirose (1990) and Murata and Hirose (1991). Glucocorticoids have been shown to suppress the production of interleukin-2 by lymphocytes which is an essential proliferative factor (Frank Blecha *et al.*, 1984). Murata and Hirose (1991) opined that serum of cattle after transportation, found to possess 28-35 kDa proteins which were not detected before transportation in these cattle, might have been related to the suppression of lymphocyte blastogenesis. However, in the present study the immunosuppressive activity was found to be reduced after 18 h of post-transport resting period and this closely agreed with reports of Murata (1989) who suggested that the factors responsible for suppressed immune response by lymphocytes been rapidly inactivated on removal of the stressor.

5.4 Effect of transport and a post-transport resting period of 18 h on biochemical parameters

5.4.1 In LDS cattle

The pre-transport blood glucose level (BGL) in LDS cattle was found to be significantly lower than values recorded at zero hour and 18 h post-transport. Numerous factors are related with hyperglycemia during stress like activation of sympathetic adrenal medullary system (Kenny and Tarrant, 1987) thereby increased glycogenolysis in liver and muscle, preceded by an elevated cortisol concentration (Kannan *et al.*, 2000), reduced glucose clearance from the circulation, and decreased tissue utilization of nutrients (Cole *et al.*, 1988). Hyperglycemia induced by transport stress is generally ascribed for the

synergestic action of cortisol, catacholamines, glucagon and thyroxine on various energy resources of body like fat depots and glycogen (Mitchell *et al.* (1988) and Ghuman *et al.* (1996)). Therefore BGL could be a useful indicator of intensity of stress experienced during transportation.

Cortisol is a useful indicator of short term stress arising from general animal husbandry practices like handling, castration etc. (Grandin, 1997) and it takes about 10 to 20 min to reach the peak level. An increased concentration of cortisol observed in LDS cattle at zero hour post-transport in the present study could be considered as a reliable indicator of short term physiological stress which was also reported by Kannan *et al.* (2000). After giving 18 h of rest, the value returned close to the pre-transport level and closely agreed with observations made by Stull and Rodiek (2000). Various stressors like handling, loading and initial stages of journey had activated hypothalamo-pituitary adrenal axis, releasing adrenocorticotropic hormone (ACTH) from anterior pituitary, resulting in the secretion of glucocorticoids from adrenal cortex (Kenny and Tarrant (1987) and Apple *et al.* (1995)). After the stressors of transport are withdrawn, the concentration of cortisol dramatically declined to the normal level probably due to the short half life (1-1½ h) of cortisol (Stull and Rodiek, 2000). As observed by Apple *et al.* (1995), an animal responds to increased physical/psychological stressors by the release of ACTH from anterior pituitary, thereby glucocorticoids from adrenal cortex as well as epinephrine and nor-epinephrine from adrenal medulla and nor-epinephrine from sympathetic nerves. These hormones serve to adapt the body to stressors

by affecting cardiovascular, energy producing and immune systems. Thus plasma cortisol and glucose levels could be used as measures of adrenal response to stress.

In the present study LDS cattle did not show any significant change in the total content of plasma protein after transport. In contrast, Stull and Rodiek (2000) reported that an increased VPRC and total plasma protein concentration could be used as indicators of dehydration due to the transport. In the present trial, the body weight loss experienced by LDS group cattle was meagre, which indicated that the fluid homeostasis was maintained by the animals thereby, overcoming dehydration with a pertinent increase in total plasma protein content.

In the present study, the high urea content of blood noticed at zero hour as well as at 18 h post-transport, could be due to the disruption of the animals' normal pattern of feeding. Following transportation, there would be a considerable amount of catabolism of body tissues leading to an increased blood urea nitrogen (BUN) content which was also reported by Kannan *et al.* (2000).

In the present investigation, considerable increase in the activity of creatine kinase (CK) was observed in cattle at zero hour and 18 h post-transport. Earlier reports also supported this observation as a mild to moderate increase in plasma CK activity after physical stress or exercise (Kannan *et al.*, 2000), starvation (Kent and Ewbank, 1986) and longer journeys (Warriss*et al.*,

1995). During the transport there is a release of CK from skeletal muscle into general circulation as a response to changes in permeability of cell membranes of fatiguing muscles.

Any significant variation in lactate dehydrogenase (LDH) activity is considered to be a delayed representation of muscle damage. In the present study, since the muscular trauma experienced by cattle of LDS group was not that much greater during transport, resulted only in a non significant variation for LDH activity during post-transport periods. This finding closely agreed with the observation reported by Cole *et al.* (1988) who opined that only those journeys lasting beyond 24 h resulted in an increased LDH activity.

5.4.2 In HDS cattle

Even after providing 18 h of rest immediately after transport for HDS cattle, except for blood glucose level (BGL), most of the other biochemical traits did not reverse and restore to the normal values. These findings pointed out that the HDS cattle did not recover to the normal state even after spending 18 h rest. Moreover, the environment and managemental conditions offered at the lairage were not optimum or conducive for an early recovery of these overstressed animals.

5.4.3 Between LDS and HDS cattle

While comparing the effect of transport between LDS and HDS cattle, it could be noted that HDS group showed significant variations in blood glucose level (BGL), blood urea nitrogen (BUN) and creatine kinase (CK) activity at zero hour post-transport. It is commonly anticipated that during transport, HDS cattle showed struggling for footing due to overcrowding, going down under foot with subsequent risk of injury, standing on fallen animals, unsuccessful attempts to change position in a full truck, stacking due to cornering at the truck while changing the speed or break etc., all expose transporting cattle to severe stress. An increased BUN content and CK activity in HDS cattle indicated that this group experienced more injuries and bruises during transport. However, an increment in cortisol concentration is a short term physiological alteration which could be predominantly noticed in only short distance journeys as reported by Kenny and Tarrant (1987), when compared to long journeys as experienced by HDS cattle screened for this study. The level of cortisol would have increased during initial stages of the journey, reaching the peak followed by an exhaustion of Zona glomerulosa of adrenal cortex response due to prolonged stressor thus showing a comparatively decreased level at the end of journey, in HDS cattle. Because of prolonged action of stress hormones for longer periods, the blood glucose level in HDS cattle was found to have significantly ($P<0.05$) increased compared to LDS cattle since the stress hormones increases circulating energy reserves like glucose, and lactate by excessive muscle glycogen degradation as suggested by Apple *et al.* (1995).

While comparing various biochemical parameters between LDS and HDS cattle at 18 h post-transport it was found that values of BUN and CK activity were found to vary significantly between groups. After 18 h post-transport the BUN content and CK activity were significantly higher in HDS cattle than the LDS cattle, which revealed that an increased tissue catabolism and greater muscle damage inflicted by transport did not restore back even after 18 h post-transport resting period. Thus the severity of damage to tissues could be perceived by these values in HDS cattle. Moreover, the managemental conditions provided for HDS cattle at the lairage were not as good enough to make them to recover from the adverse stress they experienced during transport, suggesting that adequate conducive managemental practices should be adopted at the lairage for cattle before slaughter.

5.5 Effect of transport stress on certain meat quality parameters

5.5.1 pH and glycogen content of meat in LDS, HDS and non-transported cattle at one hour and six hours post-slaughter

In the present investigation it was found that the pH attained at six hours post-slaughter was above 6.00 in all meat samples of HDS cattle whereas the same was below 6.00 in meat of non-transport and LDS cattle. The attainment of pH due to lactic acid accumulation is invariably related to glycogen content of muscle fibres. Higher ultimate pH, generally result in dark cutting condition of meat which had a negative effect in meat quality (Apple *et al.*, 1995 and Tarrant *et al.*, 1992).

In the present investigation an inverse relationship between muscle glycogen content and acidification of muscle at the end of six hour post-slaughter could be established. It has been proved that fasting reduces glycogen storage in the body which would affect postmortem acidification of the meat sample. The combined effect of fasting and transportation significantly increased muscle pH resulting in darker muscle colour as reported by Jones *et al.* (1986). An increased sympathetic arousal, adrenaline release and other stress hormones necessarily be involved in the activation of cellular level phospholylase which mediates glycogenolysis. This dark cutting condition may be primarily because of deficient muscle glycogenolysis which has got economic disadvantage including reduced organoleptic acceptance of the product and a shorter shelf life as reported by Lacourt and Tarrant (1985).

Summary

6. SUMMARY

From the present investigation it can be concluded that transportation of cattle that are meant for slaughter are being subjected to various stress factors, which were reflected with significant changes on certain haematological and biochemical parameters. The severity of transport stress brought about marked alterations in haematological and biochemical parameters in high density stocking (HDS) cattle when compared to low density stocking (LDS) group. Significant changes in haematological parameters like haemoglobin (Hb) content, blood glucose level (BGL), blood urea nitrogen (BUN) level and creatine kinase (CK) activity were observed in HDS cattle than LDS cattle.

In order to alleviate the stress during transportation it is recommended that cattle should be handled gently during loading and unloading, smooth driving of vehicle, proper stocking density (a minimum of 1.88 m^2/adult cattle), adequate ventilation in the running truck etc. are highly essential. Over and above, the time of journey and local weather conditions are also important, especially when transportation is done on very hot and humid days. Preferably transportation should be avoided in unfavourable circumstances since that causes great economic losses and gives stress to the farm animals.

From the present investigation it could be established that a post transport resting period of 18 h was sufficient to restore most of the important altered biochemical stress markers such as cortisol and blood sugar. By giving 18 h post-transport resting period, the haematological values in LDS groups

returned to pre-transport level. In case of HDS cattle even after giving 18 h of post transport resting period an increased level of BGL and cortisol concentration were maintained. Properly designed lairage with good ventilation and protection from sun along with free access to drinking water and little greens are essential for transported animals to get adequate rest before slaughter.

In the present investigation, it was found that lymphocyte blastogenic response to mitogen in the presence of autologous serum containing more than 80 ng/ml cortisol concentration was poor, suggesting the immunosuppressive effects of cortisol. Thus cattle under severe stress which have elevated circulating cortisol levels due to over response of hypothalamo-hypophyseal adrenocortical axial system, exhibited marked lymphopenia with polynucleosis and thereby increased susceptibility to diseases.

The meat obtained from animals under severe stress were with decreased glycogen levels, which result in decreased formation of lactic acid resulting in a higher pH and poor shelf life. The results of the present study revealed that while comparing with that of LDS and non-transported groups of cattle, meat samples of HDS cattle did not attain a significantly lower pH even at the end of six hours after slaughter which would easily result in dark cutting condition of the meat thereby affecting its acceptance and marketability.

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STRESS RELATED PHYSIOLOGICAL CHANGES IN CATTLE BROUGHT FOR SLAUGHTER

By

NIGIL MATHEW

ABSTRACT OF A THESIS

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Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Physiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

2002

ABSTRACT

The study was conducted in adult female cattle with an average body weight of about 250 kg which were transported for slaughter to Meat Technology Unit (MTU), College of Veterinary and Animal Sciences, Mannuthy, to evaluate the effect of transport stress on certain physiological parameters. Group I animals comprised of 12 numbers, of which six animals each were transported from Livestock Research Station (LRS), Thiruvazhamkunnu and Cattle Breeding Farm (CBF), Thumburmuzhi and they were designated as the low density stocking group (LDS) given a floor space allowance of 1.88 m^2/animal. Eight adult female cattle brought for slaughter by trucking at Corporation slaughter house, Thrissur which were given with a floor space allowance of 0.95 m^2/animal formed the Group II or high density stocking group (HDS) and eight adult female cattle brought for slaughter from University Livestock Farm, Mannuthy by walk formed the Group III category.

Body weight was recorded in LDS group cattle (group I) before and after the journey and it was observed that the percentage reduction in live weight was 2.18 per cent which was not that much appreciable due to the ideal transporting conditions provided for them. Blood samples were collected with and without anticoagulant (1) before transport, (2) immediately after the journey (zero hour post-transport) and (3) after a resting period of 18 h from LDS cattle. From HDS group, immediately after unloading (zero hour post-transport) and after 18 h of rest blood collections were done. Blood samples,

were analysed for various haematological parameters as well as for mitogen induced lymphocyte culture studies. The serum samples were also used for estimation of biochemical parameters as blood glucose level (BGL), concentration of cortisol, total serum protein, blood urea nitrogen (BUN) and activities of enzymes viz., creatine kinase (CK) and lactate dehydrogenase (LDH). Comparison of the data collected at three intervals viz., before transport, zero hour and 18 h post-transport was done within the LDS group, whereas, the comparison within HDS group was done at zero and 18 h post-transport. Comparison of the effect of transport at zero hour and a post-transport resting period of 18 h was also done between LDS and HDS groups.

It was observed that LDS group of cattle had a significant increase in volume of packed red blood cells (VPRC), haemoglobin (Hb) concentration and total leucocyte (WBC) count at zero hour. It was observed that most of the values returned to the pre-transport level after 18 h of rest. There was significant increase in biochemical parameters, like blood glucose level (BGL), concentration of cortisol, blood urea nitrogen (BUN) and creatine kinase (CK) activity immediately after transport and many of them returned to pre-transport level after 18 h post-transport suggesting that 18 h of resting period was sufficient before slaughter.

In HDS cattle higher values were recorded for the haematological traits viz. VPRC, Hb concentration and total WBC count and for biochemical parameters like BGL, concentration of cortisol, BUN and CK activity when

compared to LDS group. Most of the values did not decline to restore much, even after giving 18 h of rest indicating that these animals experienced severe stress during the journey.

In the present study it was also found that the cortisol concentration influenced lymphoblastogenic response to mitogen which was poor in the presence of autologus serum containing more than 80 ng/ml cortisol concentration as observed in both LDS (131.2 ± 18.33 ng/ml) and HDS (160.88 ± 21.87 ng/ml) groups immediately after transport which indicated the immuno suppressive property of cortisol.

Meat samples were collected from all the groups of cattle within an hour of slaughter and used for determination of pH and glycogen content at one hour and six hours post-slaughter. It was observed that meat samples of LDS and non-transported cattle showed a comparatively lower pH after six hours of slaughter which was inversely related to their glycogen content. It was also found that the meat samples of HDS cattle did not attain a significantly lower pH even at the end of six hours post-slaughter owing to the reduced glycogen content when compared to LDS and non-transported groups of cattle which could result in dark cutting condition.

It can be concluded that gentle handling, optimum transportation conditions and proper rest before slaughter in good lairage environment are required to minimize the stress in cattle brought for slaughter and to ensure production of good quality meat.