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Physiological Responses in Goats Subjected to Road Transportation under the Hot, Humid Tropical Conditions

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ABSTRACT

Animals in transport may be exposed to both psychological and physical stressors, which affect performance and health. Heat stress has been recognized as one of the commonest problems encountered during road transportation of farm animals. The influence of two different stocking densities (0.20 m^2/animal & 0.40 m^2/animal) in transit under the hot, humid tropical conditions on blood parameters and body temperature were investigated in 30 Boer does. The animals were road transported for 3 h and the control group was kept under normal conditions in the farm. Irrespective of stocking density, transportation increased rectal temperature (P<0.001), serum levels of cortisol (P<0.05) and glucose (P<0.001) and neutrophil to lymphocyte ratios (NLR) (P<0.001). Higher stocking density was more stressful to the goats based on NLR. Transportation had no significant effect on serum creatine kinase activity. Results suggested that, irrespective of stocking density, transportation under the hot, humid tropical conditions imposed a severe stress on the goats. © 2010 Friends Science Publishers

Key Words: Transportation; Stress; Stocking density; Goat

INTRODUCTION

Increasing concern regarding animal welfare is partly responsible for the considerable progress in research on livestock during transport. Animals in transport may be exposed to both psychological (restraint, handling, novelty & unfamiliar animals) and physical (hunger, thirst, injury, extreme weather conditions) stressors (Grandin, 1997). Heat stress has been recognized as one of the commonest problems encountered during road transportation of farm animals (Mitchell & Kettlewell, 1998; Zulkifli *et al*., 2010). Physiological responses to heat stress involve changes in respiration rate and blood pH, plasma concentration of ions, cardiovascular functions and hormonal changes (Silinakove, 2000; Marai *et al*., 2007). Rajion *et al*. (2001) transported goats at an ambient temperature of 29°C and noticed significant elevation in neutrophil/lymphocyte ratios (NLR) and serum glucose concentration.

According to Fazio and Ferlazzo (2003), stocking density is a major factor determining the welfare of animals in transit. High stocking densities on transport vehicles have been closely associated with greater physiological stress reactions and poorer meat quality, when compared with medium and low stocking densities (Broom, 2000). While there is substantial work on effects of road transportation in cattle, pigs and poultry (Tarrant & Grandin, 2000; Weeks & Nicol, 2000), little work has been carried out in goats, particularly under the hot, humid tropical conditions.

Adverse conditions in transported animals can be determined by observation of their behavior and measuring physiological alterations (Broom, 2000). Some commonly used physiological indicators of stress during transport are plasma levels of cortisol and leucocytes. Glucocorticoid hormones produced in and release from the cortex of the adrenal glands in response to an extremely wide range of stressors play a major role in mediating the physiological reactions. Elicitation of the adrenocortical activity is known to precede neutrophilia or hetrophilia and lymphopenia (Maxwell, 1993). Elevated NLR in transported goats has been demonstrated by Rajion *et al*. (2001) and Kannan *et al*. (2000). Increase in plasma creatine kinase activity, a useful indicator of myopathy, has been reported in goats (Kannan *et al*., 2000) following transportation. Damage to muscle leads to an increase in membrane permeability and leakage of enzymes and other markers into the blood. The purpose of the study was to determine the effect of transporting goats in high or low density group under the hot, humid tropical climate on blood parameters and body temperature.

MATERIALS AND METHODS

Animal health: The study was undertaken following the guidelines of the Research Policy of the Universiti Putra Malaysia on animal ethics.

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Animals and farm: A total of 60 healthy Boer does (4.5 months of age, mean body weight = 20.50 kg) were obtained from a commercial farm in Janda Baik, Pahang, Malaysia (3° 31' N, 101° 55' E). The climate was hot and humid with average day temperature at 35°C and night temperature at 25°C. The relative humidity fell within 70 to 90%. The goats were raised in raised slatted floor houses with zinc roofing. The dimensions of each pen of 20 animals were 8.8 m x 4.8 m. The animals were fed with Napier grass and commercial concentrate.

Experimental design and treatments: All the 60 animals, irrespective of their home pens, were randomly assigned to three groups (control & two different transport densities) with 20 animals per treatment. The effects of two transport floor spaces (0.20 m^2 per animal, HD; 0.40 m^2 per animal, LD) were investigated. The control animals remained in their home pens.

Transport vehicle, loading and journey time: An open truck with a maximum loading weight of 5000 kg was used for the study. The floor of the vehicle, measuring 5.3 m (L) × 3.1 m (W) × 2.25 m (H) was covered with wood shavings. The floor space in the truck was divided into two compartments using wooden panels. The front and rear compartments were designated for low-density (LD) and high-density (HD) treatment groups, respectively. On the day of the experiment (10:00 - 11:00 h), each transport group animal was swiftly caught, loaded into the truck and transported for about 3 h to the Animal Research Unit, Universiti Putra Malaysia, Serdang Selangor. The animals were not restrained and the journey covered villages, highways, roads with heavy traffic and traffic lights over a total distance of about 250 km with an average speed of 76 km/h. Ambient temperature during loading was 30-32°C. Food and water were withdrawn 12 h before the journey and throughout the journey.

Blood samples and recording of rectal temperature: After transportation, the animals were unloaded individually and blood samples (10 mL) were collected from each goat by venipuncture into tubes containing EDTA as an anticoagulant. The tubes were placed on ice until plasma was separated. Before centrifugation, blood smears were prepared using Wright's stain and neutrophil (N) and lymphocytes (L) were counted to a total of 100 cells (Gross & Siegel, 1983). The blood samples for cortisol assay were centrifuged and stored at -20°C until assayed, using a sensitive and highly specific RIA kit (Diagnostic System laboratories, Texas, USA). Analyses for serum creatine kinase and glucose were conducted using an automated spectrophotometer (Ultraspec ® 300; Cobas-Mira, Roche diagnostic System, CH4070 Basel, Switzerland). Immediately following blood sampling, individual rectal temperature was recorded using a digital thermometer. The probe was inserted about 3 cm into the rectum for about 1 min. The control animals which remained in the farm at Janda Baik were also subjected to sampling of blood and recording of rectal temperature.

Statistical analysis: All analyses were performed using General Linear Models (GLM) procedure of SAS (SAS Institute, 1991). A one-way ANOVA was used to analyze the data and means were separated by Duncan's multiple range test. Results were considered statistically significant ($P \leq 0.05$).

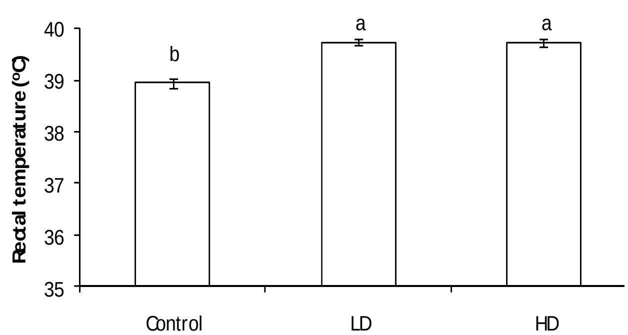
RESULTS

There was a significant (P<0.001) effect of transportation on rectal temperatures (Fig. 1). Transportation resulted in an increase in rectal temperature from 38.95°C (control) to 39.72°C and 39.74°C in LD and HD goats, respectively. The mean rectal temperatures of LD and HD goats were not significantly (P>0.05) different. The N counts of the HD and LD groups were similar but the latter had significantly (P<0.001) higher L (Fig. 2). Transport density had a significant (P<0.001) effect on NLR with the HD goats showing the highest ratios followed by the LD and control groups. Irrespective of stocking density, transportation significantly elevated serum cortisol (CORT) (P<0.05) (Fig. 3) and glucose (GLUC) (P<0.001) concentrations (Fig. 4). Both LD and HD goats had similar CORT and GLUC. Mean serum creatine kinase concentrations (CK) of control, LD and HD goats were not significantly (P>0.05) different (Fig. 5).

DISCUSSION

Although the results obtained in the present study clearly suggest that irrespective of stocking density, road transportation for 3 h under the hot, humid tropical condition was stressful to the goats the time course physiological changes during transportation are unknown. Nwe *et al.* (1996) measured plasma levels of cathecolamines and cortisol before, during and after 6 h road transportation in goats. The authors noted peak plasma concentrations of cortisol and adrenaline 1 and 2 h after the start of transit and returned to basal values 3 and 6 h after

Fig. 1: The effect of transportation on rectal temperature. [a,b] Means without a common letter differ (P < 0.001) LD = transport floor space of 0.40 m^2 per animal; HD = transport floor space of 0.20 m^2 per animal



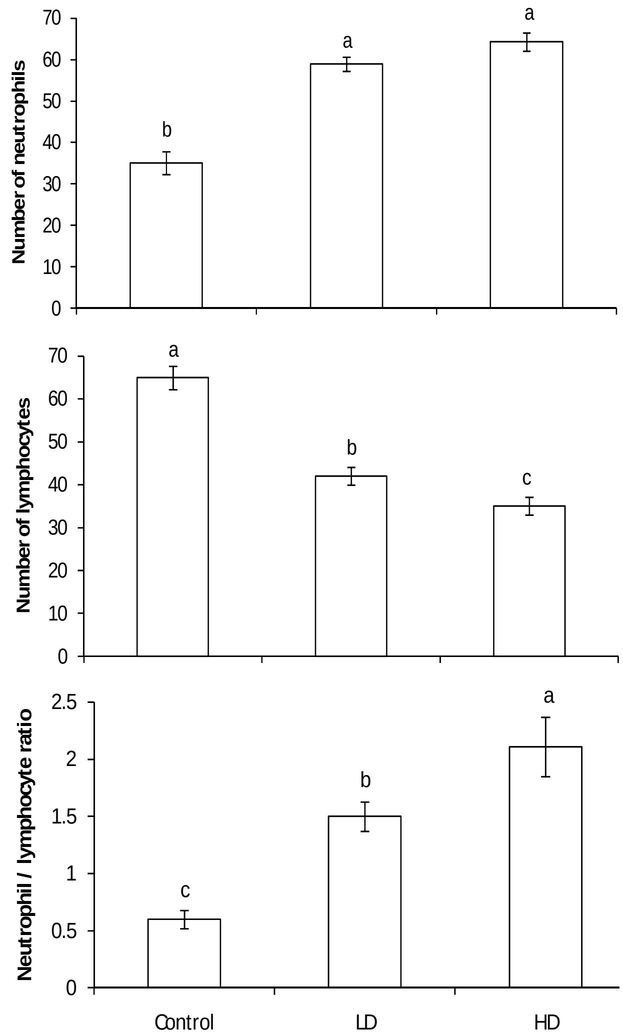
the journey, respectively. They concluded that the starting phase was the most critical period for the animals to respond to the transportation stress.

As expected transportation at ambient temperatures of 30–32°C resulted in hyperthermia among the goats. The increase in body temperature following transportation could also be attributed to stress-induced hyperthermia (Bouwknecht *et al*., 2007). The phenomenon of stress-induced hyperthermia has been reported in mammalian species when subjected to mild disturbance (Bouwknecht *et al*., 2001) and handling (Moe & Bakken, 1997). Stress-induced hyperthermia has been closely associated with an activation of the hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullary system (Groenink *et al*., 1994). Working with silver foxes, Moe and Bakken (1998) demonstrated that stress-induced hyperthermia can be blocked with anxiolytic drugs and is believed to be related to an expression of anticipatory anxiety. Hence, exposure to non-thermal stressors such as noise, vibration, motion, food and water deprivation and mixing of unfamiliar animal vibration during transit may have also elevated body temperature.

According to Mitchell and Kettlewell (1998), high ambient temperature is a major factor in the elicitation of physiological stress responses during transit in poultry. In this experiment, both LD and HD goats were equally hyperthermic following transportation. Transportation stress has been reported to alter leukocyte number in goats (Kannan *et al*., 2000; Rajion *et al*., 2001), cattle (Schaefer *et al*., 1992) and poultry (Zulkifli *et al*., 2000). In the present study, there was a dramatic elevation in NLR following the 3 h-transit. Rajion *et al*. (2001) noted an increase in NLR in goats following transportation for 1.5 h and the values remained elevated for 6 h after the transportation. The authors reported that the goats required 12 h after transportation for the NLR to return to pre-stress values. Duration of transit appears to be critical in determining the time required for the NLR to return to basal level. The NLR of goats transported for 2.5 h remained elevated 18 h after transportation.

Elicitation of adrenocortical activity is known to precede heterophilia and lymphopenia (Maxwell, 1993). In the present study, although CORT was elevated following 3 h of transportation there was no significant difference between LD and HD. Kannan *et al*. (2000) noted peak CORT in goats immediately after 2.5 h transit and a decline in values 1 h after they were unloaded and housed in holding pens. It is interesting to note that a significant difference between LD and HD was noted for NLR but not CORT. Pigs transported for 3 h did not show a significant elevation in CORT (Wariss *et al*., 1998). The authors attributed the failure to detect significant changes in CORT to the hormone relatively short half-life. Gross and Siegel (1983) suggested that heterophil to lymphocyte ratio measures a physiological change in poultry; whereas the concentration of corticosteroid in the blood is affected by many factors before physiological changes occur.

Fig. 2: The effect of transportation on number of neutrophils (top), number of lymphocytes (middle), and neutrophil/lymphocyte ratios (bottom), [a-c] Means without a common letter differ (P < 0.001), LD = transport floor space of 0.40 m^2 per animal; HD = transport floor space of 0.20 m^2 per animal



Our results concurred with those of Nwe *et al.* (1996), Kannan *et al*. (2000) and Rajion *et al*. (2001) that stress attributed to road transportation may increase serum levels of glucose in goats. Sanhouri *et al*. (1992) however, indicated that a 20-min journey elevated CORT but not blood glucose concentration. Elevation in CORT following transportation may have elicited glucoconeogenesis in which amino acids are converted to glucose and therefore blood glucose levels increased (Malheiros *et al*., 2003). Release of adrenaline and noadrenaline during the initial stage of stress may also stimulate hepatic glycogenolysis leading to hyperglycemia (Knowles & Warriss, 2000). Elevated levels of CK, a useful indicator of an increase in physical activity (Tarrant & Grandin, 2000), following

Fig. 3: The effect of transportation on serum cortisol concentration. [a,b] Means without a common letter differ ($P < 0.05$). LD = transport floor space of 0.40 m^2 per animal; HD = transport floor space of 0.20 m^2 per animal

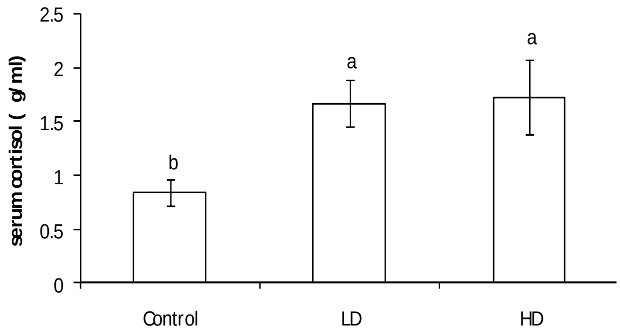


Fig. 4: The effect of transportation on serum glucose concentration. [a,b] Means without a common letter differ ($P < 0.001$). LD = transport floor space of 0.40 m^2 per animal; HD = transport floor space of 0.20 m^2 per animal

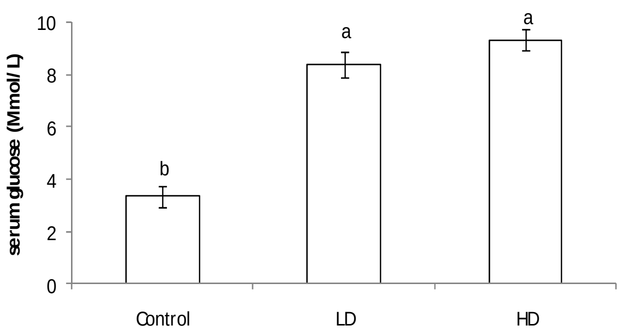
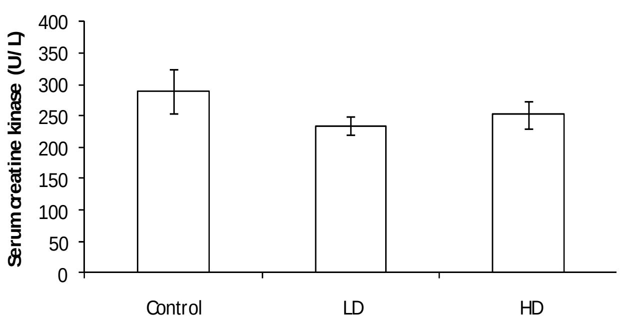


Fig. 5: The effect of transportation on serum creatine kinase concentration. Means are not significantly different ($P > 0.05$). LD = transport floor space of 0.40 m^2 per animal; HD = transport floor space of 0.20 m^2 per animal



transportation has been previously reported in goats (Kannan *et al*., 2000) and cattle (Kenny & Tarrant, 1987). Damage to muscle leads to an increase in membrane permeability and leakage of enzymes and other markers into the blood. In the present study, irrespective of stocking density, CK was not affected by transportation. The phenomenon could be owing to the length of time the goats spent standing during transportation. Kent and Ewebank (1986) indicated that in young calves, which spent more time lying down, the increase in CK is not generally noted.

One of the major factors determining the well-being of animals during transit is stocking density. Literature regarding space requirements of cattle, pigs and sheep during transit is inconsistent (Broom, 2000; Fazio & Ferlazzo, 2003; Gregory, 2007). The discrepancies could be attributed to length of the journey, weather and driving conditions and the design and type of vehicle used (Williams, 2000). Studies in sheep suggested that the ability of animals to lie down during a long trip is important for their well-being (Cockram *et al*., 1996). Information on transport density and well-being in goats is limited. According to the Animal Welfare Guidelines of Tasmania (2008), the recommended stocking density for goats with an average body weight of 20 kg is 0.17 m^2 per animal, which is larger than the space provided to the HD group in the present study. However, the stocking density recommended by the Animal Welfare Guidelines of Tasmania (2008) may not be suitable for transported goat under the tropical hot and humid conditions. Kannan *et al.* (2000) transported goats with floor spaces of 0.18 m^2 and 0.37 m^2 for 2.5 h under an ambient temperature of 35°C and noted negligible differences in plasma cortisol concentration. In the present study, as measured by NLR, the HD goats were "more stressed" than their LD counterparts. Although Kannan *et al.* (2000) transported goats under an ambient temperature of 35°C the relative humidity was not reported. The high relative humidity in the tropics may exacerbate the heat stress problem during transit. However, because both HD and LD goats had similar mean body temperatures following transportation it is unlikely that the greater NLR reaction in the former is associated with heat stress. There appears to be no clear explanation for the difference in physiological stress reaction between the two groups. It is possible that reduced mobility among the HD animals has elicited the greater leucocytic reaction.

In conclusion, irrespective of stocking density, transporting goats for 3 h under the hot, humid tropical conditions resulted in changes in body temperature, leukocyte counts and circulating levels of cortisol. These results suggest that, irrespective of stocking density, transportation under hot, humid tropical conditions imposed a severe stress to the goats.

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