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Effects of transportation positions and orientations on muscular damage of goats transported by road for 12 h during the hot-dry conditions

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The influence of road transportation position and orientation on muscular damage was evaluated in 30 goats. The animals were divided into two groups: 20 goats transported by road for 12-h during hot-dry conditions (TG) and 10 non-transported sedentary goats (SG). During the 12-h transportation period, the goats spent 7 h standing, of which 40.0% of the goats spent 4.5 ± 0.7 h standing in perpendicular direction, 30.0% spent 1.5 ± 0.5 h in parallel direction, while 20.0 and 10% spent 0.5 ± 0.1 h each in opposite and diagonal directions, respectively. Of the 5 h spent by the goats lying down, 33.6% of the goats spent 2.5 ± 0.7 and 1.5 ± 0.5 h each lying down in opposite and diagonal directions, respectively. Goats that adopted standing or lying down position in perpendicular and opposite directions during the transportation period had predominantly lower values of creatine phosphate kinase, alanine aminotransferase, aspartate aminotransferase, glucose and urea, which suggested less muscular damage and best transportation position. Lying down in opposite or diagonal orientations produced the highest (*P* < 0.05) activities of blood enzymes, and glucose and urea concentrations, which indicated high level of muscular damage and worst position. In conclusion, lying down or standing in opposite or diagonal orientations may have adverse effects on the welfare, health, productivity and meat quality of goats transported by road.

Key words: Goats, muscular damage, position and orientation, serum enzymes, stress, road transportation.

INTRODUCTION

Road transportation of animals is an inevitable husbandry practice. It is important and yet a critical period in animal production. Every year, several millions of goats, particularly the Red Sokoto goats are transported from the Northern Nigeria and neighbouring Northern countries like Niger and Chad to the Southern and other parts of Nigeria, and to countries like Benin and Togo for the purpose of slaughter for meat, skin, breeding or further production (Minka and Ayo, 2010, 2012). Such transportation covers thousands of kilometers, lasting 2 to 4 days on the road. Few studies have investigated whether long-distance road transportation of goats under

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adverse climatic conditions may compromise the well-being, and consequently, meat quality of goats (Kannan et al*.,* 2000; Minka and Ayo, 2012). Besides, the thermal aspects of the environment during the three (wet, hot-dry and harmattan) seasons in the Savannah zones of Nigeria are stressful. This is especially so during the hot-dry and harmattan seasons when the ambient temperature and relative humidity fluctuate between 20.0 to 39.4°C and 65 to 75%, respectively during the hot-dry and 12.4 to 29.3°C and 18 to 22%, respectively during the harmattan seasons (Igono et al., 1982; Minka and Ayo, 2010).

The Red Sokoto goat (RSG) or Maradi breed is characterized by a uniformly dark-red coat colour, short and horizontal ears and horns in both sexes. The males are heavier and usually weigh an average of 27 kg, while females weigh about 25 kg. The breed is extremely resistant to harsh environmental conditions, which has contributed to its great population density in the West and North Africa sub-regions. The RSGs serve as a good source of meat and high-quality skin used in leather industries, and income to the small scale farmer. They are highly productive and have a good food conversion ratio (Aganga et al., 1986; Ayo et al., 1998).

One important indicator of stress during road transportation of animals is the change in behaviour, which shows that some aspect of transportation procedure is aversive (Broom, 2000). Some behavioural changes may be signs of distress (Ayo et al., 2002, 2006; Young et al., 2012). Thus, the understanding of the behaviour of goats during transportation may provide relevant information on how transportation stress factors affect their well-being, and consequently, the meat/milk quality.

Previous investigations have estimated the effects of road transportation on different blood parameters, useful as marker of welfare condition in ruminants (Giannetto et al., 2011; Piccione et al., 2012, 2013). Blood enzymes such as creatine phosphate kinase (CPK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and metabolites have been used also as reliable physiological indices of muscle tissue injury or damage in humans and animals subjected to different stressors, including transportation stress (Ekiz et al., 2012; Cafazzo et al., 2012).

Generally, several studies have been conducted on the effect of road transportation on muscle enzymes and metabolites in cattle (Cafazzo et al., 2012; Averos et al., 2008), sheep (Galipalli et al., 2004; Ekiz et al., 2012, 2013; Zhong et al., 2012), goats (Kannan et al., 2000, 2002; Galipalli et al., 2004) and roe deer (Montane et al., 2002). Few studies have been carried out on the best travel position or orientation that is less stressful for horses (Kay and Hall, 2009; Padalino et al., 2012; Tateo et al., 2012) and cattle (Cafezzo et al., 2012; Earley et al., 2012). However, the best travel position and the effects of travel positions and orientations on serum biochemical activities of goats, transported by road have not been elucidated. Such information, if available, may enhance the health, welfare status, and consequently, meat quality of goats subjected to long-distance road transportation.

The aim of the present study was to investigate the best travel position and orientation for goats by assessing the effects of standing and lying down positions and orientations on muscular damage following long-duration transportation of goats during the hot-dry climatic conditions.

MATERIALS AND METHODS

Study area and environmental conditions

The experiment was performed at the Livestock Farm of the College of Agriculture and Animal Science, Ahmadu Bello University, Kaduna, located in the Northern Guinea Savannah zone of Nigeria during the hot-dry period of the month of April. Transportation for 12 h was conducted from Kaduna (11° 10' N, 07° 38' E) to Makera (12° 31' N, 06° 11' E), Nigeria; and from Makera back to Kaduna, covering a total distance of about 600 km. The ambient temperature (AT) and relative humidity (RH) were recorded at the study site using a wet- and dry-bulb thermometer (DTH 1, Clarke Int., Epping, Essex, UK) at 07:00, 13:00 and 18:00 h daily for 7 consecutive days before and after the transportation. The AT and RH were also recorded hourly inside the vehicle during the 12-h transportation period. After the transportation, the goats were returned to the same pen and managed as it was done before the transportation.

Animals and management

The study was approved by the Postgraduate Research Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria and conducted according to international guidelines on animal ethics (Gavinelli and Simonin, 2003). Thirty Red Sokoto goats of both sexes (aged 3 years old and weighing between 23 and 25 kg) were used. The goats were kept at night in a standard goat pen at a density rate of 1 goat/m^2 (Kannan et al., 2002). The top half of the wall of the pen was open, provided with a wire mesh for adequate ventilation. The roof of the pen was made of aluminum with a wooden ceiling, while the floor was concrete. The goats were not restrained inside the pen. Each day, the goats were grazed on a natural pasture, from 09:00 to 18:00h. Water was available *ad libitum*. On transportation day, the goats were divided into two groups: 20 transported goats (TG) and 10 non-transported sedentary goats (SG). The SG were deprived of food and water for the entire period of the transportation.

Loading, stocking density and transportation

The loading and transportation of the goats were carried out humanely in accordance with the EU legislation governing transport of live animals, Directives 19/628 EEC amended by Directives 95/29/EEC (Earley et al., 2012). Briefly, the floor of the vehicle was covered with wood shavings with a thick rubber mat placed on top. The loading of the goats was done individually by two persons. In a relatively calm condition, one person easily caught one goat randomly at a time and calmly lifted it up by supporting the goat with two hands from the abdomen. The goat was then handed to the other person, already inside the vehicle. The goats were stocked at a rate of 0.3 m^2 per animal (Kannan et al., 2000; Minka and Ayo, 2012) in a standard articulated Bedford, open pickup van, popularly used in the Guinea Savannah zone of West Africa for transportation of small ruminants. The journey started around 08:00 h and was terminated at 20:00 h. The vehicle used for the journey traveled with an average speed of 50 km/h and was driven by two licensed drivers in a similar pattern (that is not exceeding the average speed and by gentle negotiation of corner and application of breaks) on the same type of road. During the journey, the vehicle had four stop-overs; at veterinary and police control posts, and each stop-over time took about 10 to 15 min. In adherence to EU legislation on transport of live animals, the goats were rested for 1 h after an 8-h transportation period (Gavinelli and Simonin, 2003). During the resting period, the vehicle was parked in a shade to avoid direct effect of heat from sun. The goats were not fed or watered during the resting period (Richardson, 2002; Minka and Ayo, 2012).

Behavioural measurements

The behavioural events of the goats were recorded visually as earlier described (Altmann, 1974; Das et al., 2001) with little modification in timing, and using a digital camera (Sony, Minato-Tokyo, Japan) mounted at an angle in each truck to complement any visual lapses. Briefly, four trained observers recorded the behaviours; each observer stood at each of the four corners of the truck. During 12 h of transportation, each observer recorded behaviours of 5 goats on specially designed cards. The observers recorded standing and lying down positions and orientations of the goats after loading, before the start of driving and during the 12-h transportation period. The observers scanned each individual current activity (standing or lying down in perpendicular, parallel, opposite or diagonal orientations) at each 10 min interval, giving a period of 30 min of direct observation made for every 1 h. The percentage number of goats and the amount or percentage of time that individuals devoted to various activities (standing and lying down positions and orientations) were noted. The times spent in different positions and orientations were estimated for every 10 min using the 'instantaneous' sampling method described by Altmann (1974). Similarly, the percentage number and time the SG spent in standing and lying down positions were also assessed by two observers during the 12-h fasting period.

Blood analysis

A week before transportation (at 8:00 h and 20:00 h), before loading at 8:00 h and at the end of the journey, immediately after unloading, at 20:00 h, blood samples were collected from each goat in both groups by jugular venepuncture using #21 scalp vein set (Jiangsu Kanghua Medical Equipment Co. Ltd., Kiangsu, China) into non-heparinized test tubes. The blood samples were centrifuged at 1500 × g for 10 min after clotting at room temperature (about 22 to 26.7°C). The plasma obtained was immediately analysed for the assessment of activities of ALT, AST and CPK using an automated analyzer (COBAS MIRA, Roche, Nutley, NJ, USA), while glucose and urea were assessed using an autoanalyzer (YSI 2300 STAT Plus, Yellow Springs, OH USA) at the Clinical Pathology Laboratory, Ahmadu Bello University, Zaria, Nigeria.

Statistical analysis

The AT and RH values recorded during the journey and in the goats' pen were compared using Student's *t*-test. The differences among standing or lying down positions and orientations (perpendicular, parallel, opposite and diagonal) of the animals during transportation were analyzed using Chi-square. The Wilcoxon-signed rank test was used to compare the hourly rates of behavioural categories observed at each sampling time, and the behaviour of animals under the different conditions (before, during and after the journey). Blood parameters were analysed using repeated-measures analysis of variance according to the procedure of the Generalized Linear Model of SAS (2006). Independent variables were the positions and orientations, time of observations and the interactions between these variables. Tukey's *post-hoc* test was used for statistical multiple comparisons, taking $P < 005$ to be significant.

RESULTS

Environmental conditions

The environmental conditions recorded inside the vehicles during the transportation periods and in the pen are shown in Figure 1. Before and after transportation, the AT and RH values fluctuated between 26.2 to 37.7°C and 50.0 to 80.0%, respectively; while during transportation, the AT and RH inside the vehicle fluctuated between the values of 28.2 to 40.1°C and 58 to 79%, respectively. The AT values recorded inside the vehicle, especially during the afternoon hours, were significantly ($P < 0.05$) higher than those recorded in the pen where the SG were kept, and also higher than ($P < 0.05$) the corresponding values recorded before and after transportation. There was no significant difference in the RH value recorded inside the vehicle and in the goats' pen (Figure 1).

Behaviour of goats

The first 30 min to one hour of the journey was characterized by frequent changes in standing positions, with parallel orientation taking precedence (not shown in Figures or Tables). However, as the journey progressed, the behaviour of the goats became stable with little alteration when the vehicle negotiated bends or applied breaks. The hourly fluctuation in standing behaviour of the TG and SG are shown in Figure 2. The first half of the transportation period was characterized by standing behaviour. The time spent by TG in standing position during the 12-h transportation period was significantly ($P < 0.05$) higher than that spent in standing position by the SG. Table 1 shows the time spent and percentage number of goats that exhibited different positions and orientations during the transportation period. Of the 12-h

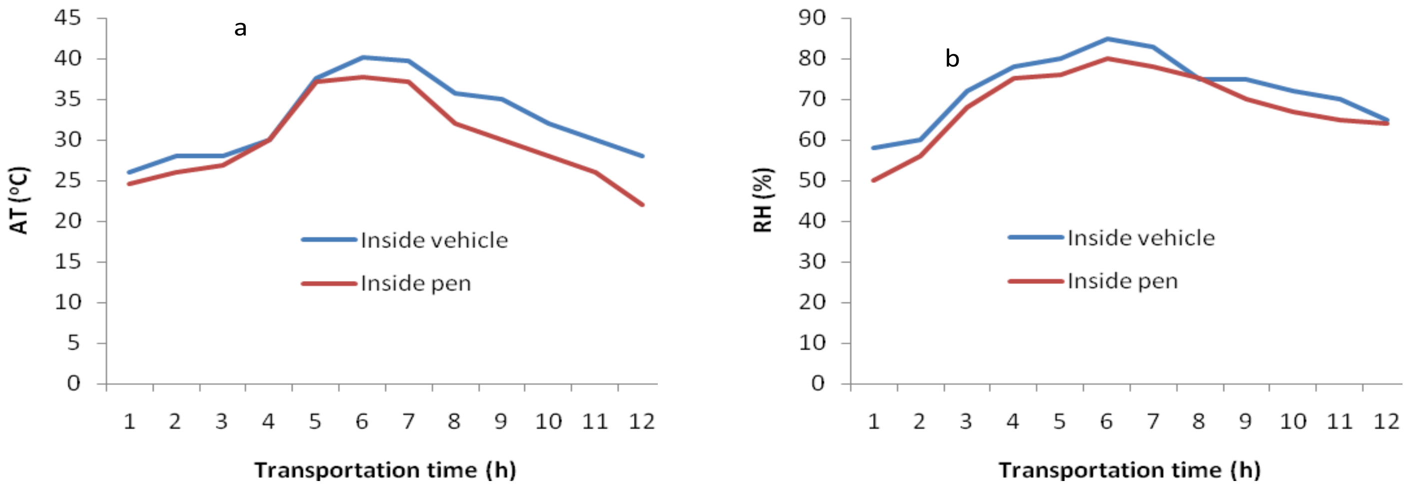

Figure 1. (a) AT (°C) and (b) RH (%) fluctuations recorded inside the vehicle and pen during the 12 h of road transportation.

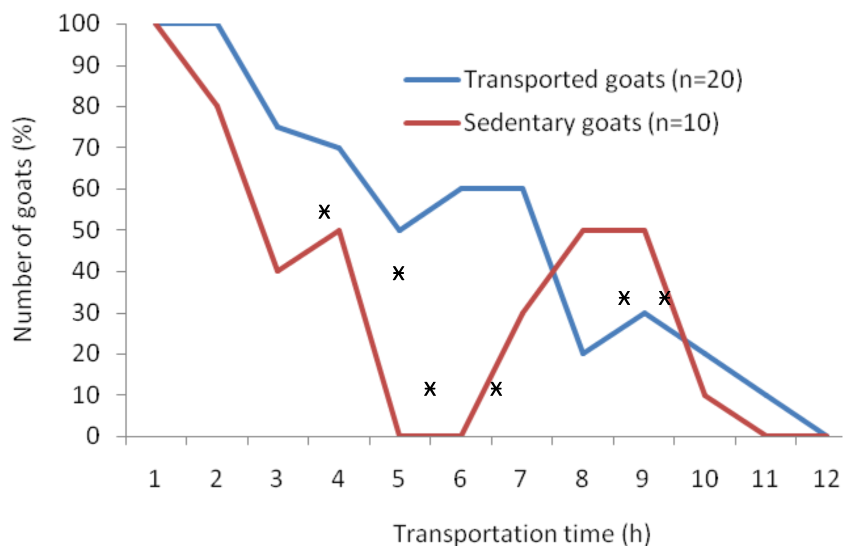

Figure 2. Changes in standing behaviour of transported and sedentary goats during 12 h of road transportation period.
*P < 0.05 vs. transported.

transportation period, the goats spent 7 h standing, of which 40.0% of the goats spent 4.5 ± 0.7 h standing in perpendicular direction, 30.0% spent 1.5 ± 0.5 h in parallel direction, while 20.0 and 10% spent 0.5 ± 0.1 h each in opposite and diagonal directions, respectively. Of the 5 h spent by the goats lying down, 33.3% of the goats spent 2.5 ± 0.7h and 1.5 ± 0.5 h each lying down in opposite and diagonal directions, respectively (Table 1).

Plasma enzyme activities and metabolites

The concentrations of plasma biochemical activities and metabolites obtained in SG were not different from the base-line values. Immediately after transportation, mean activities of CPK, AST, ALT, and glucose and urea concentrations recorded in TG were elevated (*P* < 0.05) over base-line, and over the values recorded in SG (Table 2).

Table 1. Mean time spent and percent number of transported goats (**n =** 20) that exhibited different positions and orientations during 12 h of road transportation.

Orientation	Position			
	Standing	Lying down	Standing	Lying down
	Mean time (h)		Percent (%) number of goats	
Parallel	1.5±0.5^{ax}	0.5±0.9^{bx}	30^{ax}	16.6^{bx}
Perpendicular	4.5±0.7^{ay}	0.5±1.6^{bx}	40^{ay}	16.6^{bx}
Opposite	0.5±0.1^{az}	2.5±0.7^{by}	20^{az}	33.3^{by}
Diagonal	0.5±0.1^{az}	1.5±0.5^{bz}	10^{az}	33.3^{by}
Total	7.0	5.0	100	100

^{ab} = Values under each separate heading with different superscript letters along the same row are significantly different (P<0.05).$^{\mathbf{xyz}}$ **=** Values under each separate heading with different superscript letters along the same column are significantly different (P<0.05).

Table 2. Effects of 12 h road transportation and food deprivation on serum biochemical activities of transported (n =20) and fasted sedentary goats (n =10).

Variable	Measurement time				
	Group	Base-line	Pre-transportation	Post-transportation	Significance
CPK (IU/L)	TG	87.6±2.7	86.8±5.5	97.1±4.5^{b}	*
	SG	86.6±2.5	84.5±3.5	88.7±5.5^{a}	ns
	Significance	ns	ns	*	-
AST (IU/L)	TG	36.8±3.1	38.9±4.5	54.7±6.1	*
	SG	38.7±2.2	38.7±2.5	40.2±1.2	Ns
	Significance	ns	ns	*	-
ALT (IU/L)	TG	58.2±4.0	60.1±1.8	86.8±6.2	*
	SG	58.8±5.0	58.8±1.8	60.0±3.8	ns
	Significance	ns	ns	*	-
Glucose (mg/dl)	TG	185.7±8.4	184.0±8.9	234.5±9.4	*
	SG	187.2±11.5	184.0±10.2	190.2±8.2	ns
	Significance	ns	ns	*	-
Urea (mmol/l)	TG	3.4±0.1	3.2±0.5	3.0±0.7	ns
	SG	3.5±0.2	4.0±0.7	3.5±1.5	ns
	Significance	ns	ns	ns	-

CPK = creatine phosphate kinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase. ns = P>0.05; ***** = *P*<0.05.

The increase in plasma biochemical activities and metabolite concentrations was lower in TG that spent more time standing and lying down perpendicular to the direction of the vehicle movement, compared to values obtained in goats that adopted other positions (Table 3). Goats that spent more time standing and lying down in opposite or diagonal direction had predominantly higher values in all the enzymes and metabolites studied compared to those that stood or lay down in perpendicular or parallel orientations (Table 3).

DISCUSSION

The AT and RH (which are measures for temperature - humidity index, THI) values obtained inside the vehicle during the transportation, especially during the hot afternoon hours of the day, may induce heat stress because the values were outside the reference thermo-neutral zone of 22 to 35°C and 58 to 65%, respectively, established for goats in the tropics (Igono et al., 1982; Richardson, 2002). Such increase in environmental

Table 3. Effects of standing and lying down positions and orientations on serum biochemical activities of goats (n = 20) transported for 12 h during the hot-dry conditions.

Variable/position	Orientation			
	Perpendicular	Parallel	Diagonal	Opposite
CPK (U/l)				
Standing	91.2±2.5^{ax}	94.5±5.5^{ax}	97.6±2.5^{ax}	98.5±4.8^{ax}
Lying down	92.1±4.5^{ax}	99.6±4.7^{ax}	101.4±8.2^{ax}	102.5±7.9^{ax}
AST (U/l)				
Standing	40.2±2.8^{ax}	50.4±1.5^{ay}	58.1±-*5.0^{ay}	58.2±4.0^{ay}
Lying down	42.5±3.5^{ax}	55..6±2.0 ^{ay}	65.4±5.2^{ay}	67.5±5.0^{ay}
ALT (U/l)				
Standing	74.6±2.7^{ax}	82.1±4.7^{ax}	90.2±3.0^{ay}	90.8±7.5^{ay}
Lying down	79.2±3.5^{bx}	89.4±5.5^{by}	95.6±5.5^{ay}	95.2±6.8^{ay}
Glucose (mg/dl)				
Standing	198.4±12.5^{ax}	200.5±15.3^{ax}	245.1±10.5^{ay}	260.2±10.8^{ay}
Lying down	211.5±10.5^{bx}	227.4±11.5^{bx}	245.4±12.5^{ay}	270.0±15.5^{ay}
Urea (mmol/l)				
Standing	3.5±0.5^{ax}	3.8±0.7^{ax}	4.2±0.5^{ax}	4.2±0.6^{ax}
Lying down	3.5±0.5^{ax}	3.9±0.2^{ax}	4.4±0.2^{ay}	4.5±0.8^{ay}

CPK = creatine phosphate kinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase. ^{ab} = Values under each separate heading with different superscript letters along the same row are significantly different (P<0.05). ^{xy} = Values under each separate heading with different superscript letters along the same column are significantly different (P<0.05).

temperature is characteristic of the hot-dry season in the zone of study and has been shown to induce oxidative stress, which consequently reduces the coping ability of the animals (Minka and Ayo, 2012). The unfavourable environmental conditions acting on the animals in this study have been considered a risk factor for animal welfare (Minka and Ayo, 2012), which often results in their death or compulsory slaughter (Ibironke et al., 2010; Minka and Ayo, 2012). Thus, management factors toward alleviation of the adverse effect of environmental conditions on transported vehicles should be employed, especially if the animals are to be transported during the hot afternoon hours of the day. The standing position observed in the majority of the goats suggested that the goats preferred standing position during transportation period and only lay down when exhausted and fatigued, which set in after 7 to 8 h of transportation. This finding was supported by the results obtained in Indian goats transported for 50 min by Das et al. (2001).

The results of standing orientations during the transportation showed that the majority of the goats that exhibited standing positions spent more time in perpendicular orientation to the direction of the vehicle movement. The present result disagrees with the finding of standing orientation parallel to the direction of vehicle movement reported in Indian goats transported by road for only 50 min (Das et al., 2001). The difference in the orientation may be due to variation in the journey duration. Similar result on standing position parallel to the vehicle as reported by Das et al. (2001) was observed during the first hour of transportation in the present study. However, as the journey progressed the goats changed their positions from parallel to perpendicular. In transported cattle, the predominant standing orientations observed were parallel or perpendicular to the direction of movement (Eicher and Morrow-Tesch, 2000; Eicher, 2001; Nanni et al., 2003).

In transported horses, the most preferred and less stressful standing orientation was opposite to the direction of vehicle movement (Padalino et al., 2012), which is in contrast to the present result. Cafazzo et al. (2012) showed that provision of adequate space for animals during transportation makes the choice of a particular standing orientation unnecessary. The reason for the choice of standing in perpendicular orientation by the goats in the present study may be due to increased demand in oxygen as the journey progressed. Thus, the goats apparently adopted this orientation so that their nostrils will be exposed to more air as they inclined their heads in perpendicular direction. This may require further investigation. The observation that the TGs spent more time in standing position during transportation than the time spent by the SG suggested that the transportation affected standing stereotype of the goats.

The increase in the biochemical activities of AST, ALT and CPK observed over transportation suggested that transportation increased muscle cell permeability and injury, which resulted in the leakage of the enzymes. The result showed that transportation may cause muscle break-down and bruising, similar to those obtained in transported cattle (Averos et al., 2008; Cafazzo et al., 2012), sheep (Zhong et al., 2011; Ekiz et al., 2012, 2013), goats (Kannan et al., 2000; Galipalli et al., 2004) and roe dear (Montane et al., 2002). The increase in plasma glucose during the post-transportation period may be due to increase in glycogenolysis, stimulated by increased secretions of catecholamine and glucocorticoid hormones, which are under the control of the sympathetic nervous system (Ekiz et al., 2013). Thus, during the stressful transportation period, the sympathetic nervous system was apparently activated to trigger the secretion of hormones, responsible for the stimulation of glycogennolysis for more production of glucose from the liver and muscles into the systemic circulation (Rajion et al., 2001; Kannan et al., 2002; Averos et al., 2008). The increase ($P < 0.05$) in plasma urea indicated an increase in protein break-down, apparently due to excess fatigue, increase in cortisol concentration and prolong food deprivation, which cause break-down (catabolism) of some proteins and nucleic acids in muscles during stressful transportation conditions (Kannan et al., 2002; Ekiz et al., 2013).

The lower values of enzymatic activities, though not statistically significant, recorded in goats that spent more time in standing position, as compared to those that lay down, demonstrated that these goats were less stressed, and thus, suffered less muscular damage. It is expected that longer duration of standing should produce greater adverse effect on muscles of the goats, but surprisingly, this was not so. The result suggested that standing and maintenance of balance inside transpor-tation vehicle may not be a major problem for goats, apparently due to their light live-weight. Furthermore, the fact that the goats were reared under the extensive management system and were used to grazing in different terrains, climbing of shrubs and wall fences, apparently, facilitated their ability to maintain stable and long duration of stan-ding position, with little stress during the transportation. Such adaptational abilities were reported in transported cattle and goats reared under the extensive management system (Kannan et al., 2002; Minka and Ayo, 2012). The significant decline in standing position observed from the 8th h of the transportation showed that journeys exceeding exceeding 8-h in goats were stressful and resulted in muscular fatigue, as evidenced by the large number of goats that lay down from the 8th h of the transportation period.

Furthermore, the fact that the physiological indices of muscular damage recorded in the TG after the transportation were above the values recorded in SG, strongly suggested that the increase in the values was mainly due to transportation conditions, rather than food and water deprivation. The insignificant increase in enzyme activities and glucose and urea concentrations recorded in SG after the 12 h of food and water deprivation supported the findings of Schoen (1968) and Aganga et al. (1986), who showed that food and water deprivation in goats for up to 72 h had little or no effect on their basic physiological parameters.

The lower activities of enzymes, and concentrations of glucose and urea obtained in goats that spent more time standing or lying down in perpendicular orientation demonstrated that those goats suffered less stress as compared to their counterparts goats that spent time standing in other different orientations. The result showed that perpendicular orientation was the most preferred standing orientation during long duration of road transportation of goats, reared under the extensive management system under hot-climatic conditions. The worst lying down orientation was opposite or diagonal to the direction of travel. This was evidenced by higher physio-logical indices of stress recorded in goats that adopted lying down position in opposite or diagonal orientations. The result of the present study, for the first time, demonstrated the adverse effects of standing and lying-down positions and orientations on activities of plasma enzymes, concentrations of glucose and urea of goats, subjected to long-distance road transportation under hot-climatic conditions. Therefore, management strategies towards alleviation of road transportation stress in goats should include vehicle modification that would allow the animals to stand or lay perpendicularly to the direction of vehicle movement.

Conclusions

Standing or lying down perpendicularly during road transportation of goats induced less muscular damage, while lying down in opposite or diagonal direction to the vehicle movement induced greater muscular damage, which may adversely affect the welfare of the goats. Perpendicular orientation may be the best travel position for goats transported by road under adverse environmental conditions.

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