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Effect of vitamin C, electrolyte, and jaggery on transportation stress in different seasons on biochemical parameters of goats

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Abstract The present study evaluated the seasonal effects of transportation of goats (Alpine x Beetal) at different flocking densities, supplemented with Vitamin C in group I, Vitamin C + Electrolyte in group II and group III Jaggery, three days before transport of animal, during winter and hot-humid seasons. The goats were selected from LRC, NDRI Karnal, and were between 10-12 months old. Each group has been 25 goats, divided into high (15) and low (10) flocking densities, with an average speed of 25 Km/h transported for 8h. All the animals were kept off-feed and deprived of water during transportation. Blood samples were taken just before transportation, immediately after transportation, 6hrs, 12 hrs, 24 hrs, and two days post transportation from all three groups. The blood samples were further analyzed to estimate different biochemical parameters such as Glucose, GPx, and SOD. Pre-transportation values of glucose, GPx, and SOD were lower ($P<$ 0.05), followed by a maximum increase in values after unloading in all the groups and both seasons. The values continued to decline with subsequent hours after post transportation till 24 hours. Vitamin C, Vitamin C + Electrolyte, and Jaggery supplementation helped reduce transport stress, but the combination of Vitamin C + Electrolyte was more effective in reducing the transport stress in the goats.

Keywords: flocking density, glutathione peroxidase, superoxide dismutase, temperature humidity index

1. Introduction

The road transport is known to be dangerous, leading to increased morbidity and death of animals. During the transport operation, animals are subjected to several stressors, such as inappropriate handling of loading and unloading, feed and water deprivation, long journey times, overcrowding, noise and vibration of the vehicle, and harsh environmental conditions, are well known to all having the potential to significantly weaken animal resistance to diseases by depriving cellular and humoral immunity (Fazio and Ferlazzo 2003). It includes handling, loading, and unloading, which is the most challenging time of the journey (Minka and Ayo 2008). Most research on the transport of goats is performed in temperate regions around the world (Rajion et al 2001), and there are still limited measures to alleviate transport stress for goats (Galipalli et al 2004 and Minka and Ayo 2007b). Transportation of Goat has recently expanded to include goat meat and skin worldwide (Minka and Ayo 2007). Higher mortality, morbidity, loss of live mass, malnutrition, and poor quality of meat result in significant losses because of hot-dry and hot-humid seasons (Minka and Ayo 2007a,b).

Stocking density is a primary determinant of animal welfare during transit (Fazio and Ferlazzo 2003). In contrast to medium and low stocking densities, higher transport stocking densities of the vehicle were closely linked to higher physiological stress and lower meat quality (Broom 2000).

Glucose measures the blood sugar level, an energy source for each cell. High glucose levels can occur during a stressful situation. Low glucose levels occur when an animal does not eat or when there is a severe bacterial infection in the bloodstream. Plasma glucose is a commonly used physiological stress indicator during transportation (Broom 2003; Tadich et al 2005; Lopez et al 2006; Averos et al 2008). Transport stress has been identified as causing an increased level of glucose in the plasma due to liver disintegration of glycogen or the depletion of the skeletal muscle glycogen reserves (Kannan et al 2000; Tadich et al. 2005; Averos et al 2008). Animals, when transported through long distances, are submitted to intense stress, as well as water and food deprivation, leading to homeostatic changes, thus affecting glycogen concentrations and muscle pH (Burns et al 2014). Ponter et al (2003) studied the effects of repeated transportation in pregnant goats and their offspring; they reported that glucose concentrations were higher in transport goats compared to control goats on all days of transport ($P <$ 0.01). An effect of the day of transport was observed on the glucose response ($P <$ 0.01), with an increase from day 1 to day 9. Kumar et al (2003) studied the effect of road transport stress on the blood profile in the Mecheri breed of sheep. They found

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that plasma cortisol, protein albumin, globulin, and glucose in sheep transported for 180 km and those transported for 410 km significant increased these parameters immediately after transportation. However, the sheep had plasma cortisol and glucose decreasing significantly just to slaughter, and the values remained higher than those recorded pre-transportation. During 2.5 h road transportation of goats, Kannan et al (2000) reported an increase in glucose concentration. This remained high for the first 3 h after transportation and then decreased at 3 h. Nwe et al (1996) reported that plasma glucose concentrations returned to baseline levels at 3 h after a 6 h journey in male Japanese goats. Kannan et al (2003) found that short-term preslaughter transport caused significant changes in the stress responses of goats, as evidenced by an increase in cortisol, glucose, and non-esterified fatty acid plasma concentration. Still, the magnitude of cortisol and glucose responses to stressor treatment was greater in older goats than younger ones. During the transportation of cattle, glucose concentrations increased from approximately 4.5 to 5.5 mmol/l after journeys of 21, 26, and 31 h, and there was a marginally greater increase in the cattle transported for 14 h from approximately 4.5 to 6.0 mmol/l. Glucose concentration remained constant at approximately 4.6 mmol/l in the 14, 21, and 26 h groups but increased by about 1 mmol/l in a group of cattle transported for 31 h (Knowles et al 1999). An increase in plasma glucose concentration is mainly due to glycogenolysis associated with the increase in catecholamines and glucocorticoids released during transportation stress (Tadich et al 2005).

Zulkifli et al (2010) reported that irrespective of stocking density, transportation significantly elevated serum cortisol ($P<0.05$) and glucose ($P < 0.001$) concentrations. LD (0.40m²/animal) and HD (0.20m²/animal) goats had similar cortisol and glucose concentrations. Teke et al (2014) found that glucose concentrations in the high stocking density group lambs increased. Miranda-de la Lama et al (2011) found that lambs transported on unpaved roads had significantly higher plasma glucose levels than those transported on paved roads. Tarrant et al (1992) reported elevated plasma glucose levels associated with increasing stocking density in cattle. A study by Rajion et al (2001) revealed that irrespective of the time of transportation, goats exhibited a marked ($P < 0.05$) rise in plasma glucose concentrations after transportation but showed recovery within 6h, and values remained constant after that. A study on camel transport by Khasmi et al (2013) showed a significant increase ($P < 0.05$) in glucose concentration post-transportation.

Physical and psychic exertions occurring during road transportation of animals disrupt their homeostasis and metabolism and, consequently, increase the activity of enzymes and hormones (Ayo and Oladele 1996; Mstl and Palme 2002; Adenkola et al 2009). Superoxide dismutase is a metalloenzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. It is important in the antioxidative defense mechanism and protects against lipid peroxidation (Halliwell and Chirico 1993; Miller et al 1993). The higher activity of erythrocyte SOD is due to higher summer temperatures in cattle (Bernabucci et al 2002). Studies on Beetal goats showed that erythrocytic SOD activity in summer-stressed goats was significantly higher than in the pre-summer values. The up-regulation of erythrocytic SOD activity indicated that the goats experienced thermal stress (Kumar et al 2010). Superoxide dismutase activities for goats subjected to 7 h of transportation in the hot-humid tropical condition decreased through the transit period, with the lowest values obtained immediately after transport (Polycarp et al 2015). Al-Badwi et al (2012) have reported a significant ($P < 0.05$) decrease in SOD levels in goats' post-transportation. A significant reduction in the plasma SOD activity compared to baseline values was reported in horses transported for 12 h (Onmaz et al 2011), beef cattle (Celi et al 2010), and goats (Kannan et al 2007).

The seleno-enzyme glutathione peroxidase, as one of the primary antioxidant enzymes, contributes to the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides from oxidative damage caused by free radicals (Flohe et al 1973). Increased activity of the GSH-Px was reported in cattle due to higher temperatures during summer (Bernabucci et al 2002). Nazifi et al (2009) reported that the mean concentration of glutathione peroxidase activity (297.86 ± 25.68 U/g Hb) in basal pre-transport conditions showed a significant increase of 24 h after arrival. Polycarp et al (2015) have reported that the reduced GSH activities in goats subjected to 7 h of transportation in the hot-humid tropical condition decreased sharply and significantly ($P < 0.05$) from the baseline value of 79.4 to 53.6 µM immediately after the following transportation. The present study investigated the comparative ameliorative effects of vitamin C, vitamin C + electrolyte, and jaggery following loading and unloading after transport on biochemical parameters in goats raised extensively in a tropical country during the hot-humid and winter seasons.

2. Materials and Methods

2.1. Experimental site and thermal environment conditions

The location is at an altitude of 250 m above sea level, at 29º42' N latitude and 77º02' E longitude. The average recorded temperature will reach 45 °C in the summer, and the minimum temperature in winter is 3.5 to 4 °C. The annual precipitation is about 700 mm. The experiment was conducted in hot-humid season (September-October) and winter (December-January).

2.2. Animals and Experimental plan

Crossbred 10-12-months old goats (Alpine x Beetal) were divided into three groups obtained from LRC, NDRI Karnal. Groups I, II, and III consisted of 25 goats divided into high (15) and low (10) flocking densities of 0.14 mt^2 (lfd) and 0.20 m^2 (hfd)

per goat of 20-25 kg, respectively. Continuous 8 hours travel for three days in a tractor-trailer with a minimum speed of 25 km/h. All the animals were kept off-feed and deprived of water during the journey.

Group I goats was orally fed vitamin C at 180 mg/kg.bwt/day/animal, Group II goats was fed with vitamin C + electrolyte (180 mg/kg.bwt/day/animal of vitamin C + 7g/animal/day Electral powder)and Group III goats was fed jaggery at dose of 200g/animal/day orally with limited concentrate, three days before to start of the experiment.

Electrolyte, which is based on W.H.O formulae manufactured by FDC limited tradename - Electral is used which supplies electrolytes in the following concentrations (Table 1).

Jaggery is the sugarcane-based traditional Indian sweetener. It is easily accessible to rural people and nutritious. Over 70 percent of the world's total production is produced in India. Jaggery is rich source of sucrose besides containing a good amount of vitamins and minerals (Singh, 2013), as shown in Table 2.

Table 1 Composition of Electral powder.

Electrolytes	mOsmol / Litre
Sodium	75
Potassium	20
Chloride	65
Citrate	10
Dextrose	75
Total Osmolarity	245

Table 2 Composition of Jaggery (per 100gram).

Carbohydrates (g)		Vitamins (mg)	
Sucrose	72-78	Vitamin A	3.8
Fructose	1.5-7	Vitamin B1	0.01
Glucose	1.5-7	Vitamin B2	0.06
Minerals (mg)		Vitamin B5	0.01
Calcium	40-100	Vitamin B6	0.01
Magnesium	70-90	Vitamin C	7.00
Phosphorous	20-90	Vitamin D2	6.50
Sodium	19-30	Vitamin E	111.30
Iron	10-13	Vitamin PP	7.00
Manganese	0.2-0.5	Protein	280 mg
Zinc	0.2-0.4	Water	1.5-7 g
Copper	0.1-0.9	Calories	312

2.3. Sampling Procedure

The focal animals were housed at the same place five days before the start of the transportation. Blood samples were taken just before transportation, immediately after transportation, 6hr, 12 hr, 24 hr, and two days post transportation in heparinized vacutainer tubes and immediately placed in icepacks and brought to the laboratory. Then blood samples were centrifuged at 2500 rpm for 20 minutes to separate plasma. Then plasma samples were stored in 2ml micro vials and labeled and stored at -20 ^{o}C until they were analyzed for biochemical parameters.

2.4. Biochemical parameters

2.4.1. Glucose

Glucose was estimated in plasma samples using GOD-PAP Trinder's kit method purchased from Avecon Healthcare Pvt.Ltd. In the presence of enzyme peroxidase glucose oxidase (GOD) oxidizes glucose to gluconic acid and hydrogen peroxide. Released hydrogen peroxide is coupled with phenol and 4-amino antipyrine (4-AAP) to form a colored dye. Colored dye intensity was measured at 505 nm. Kit reagents were prepared and stored as per the instructions provided with the assay kit.

2.4.2. Superoxide Dismutase (SOD)

Superoxide Dismutase was estimated by the quantitative colorimetric method using the EnzyChromTM Superoxide Dismutase Assay kit (ESOD)-100) from Bioassay Systems company.In this assay, superoxide (O2·) is provided by a catalyzed reaction by xanthine oxidase (XO). O2· reacts with a WST-1 dye to form a colored product. SOD scavenges the O2·; thus, less O2· is available for the chromogenic reaction. SOD activity in a sample is used to determine the color intensity (OD440nm).

2.4.3. Glutathione Peroxidase (GPx):

Glutathione peroxidase activity was estimated by the quantitative colorimetric method using the EnzyChromTM Glutathione Peroxidase Assay kit (EGPX)-100) from Bioassay Systems company.In the enzyme-coupled reactions,GPx activity estimation is based on the direct measurement of NADPH consumption. The measured decrease in optical density is directly proportional to the enzyme activity of the sample at 340nm. GPx catalyzes the following reaction with glutathione reductase (GR):

$$2GSH+ROOH \xrightarrow{GPx} GSSG +ROH+H_2O$$

$$GSSG +NADPH \xrightarrow{GR} 2GSH+NADP^+$$

2.5. Environmental parameters

Such parameters suggest that the study was conducted in the Indian hot-humid climate. Minimum and maximum ambient temperatures, dry bulb, and wet bulb temperatures were recorded with respective thermometers for the microenvironment of the experimental goats inside the shed and vehicle at the time of sampling and recording physiological responses. The THI was calculated from the dry bulb and wet bulb temperatures using the equation: THI = 0.72 x (Cdb + Cwb) + 40.6 (McDowell et al 1976). Daily relative humidity was determined by the difference in temperature of dry bulbs and the temperature of wet bulbs.

2.6. Statistical Analysis

Data analysis was performed using NDRI-Licensed SAS 9 software. Mean values at different sampling times were compared with respective basal mean values of each group using one-way ANOVA with post-test as Dunnets multiple comparisons.

3. Results

Meteorological results were 77.14 and 58.74, respectively, in the September-Oct and Dec-Jan THI. In Karnal, India, this indicates a hot-humid (HH) and winter seasons. The average values of biochemical parameters estimated in treated groups (group I, group II and group III) of goats at low and high flocking density during winter and hot-humid seasons have been presented in Tables 3 to 5. The analysis of variance in Table 6 was followed by their graphical representations (Figures 1 to 3), and there was a significant difference ($P < 0.05$) between seasons, between density, and between groups.

3.1. Glucose (mg/dL)

Pre-transportation glucose levels were lower in all three groups in both seasons. In group I, in winter and hot-humid seasons, higher values of glucose ($P < 0.05$) were observed in goats with high flocking density in comparison to low flocking density (Table 3). The maximum values of glucose levels were recorded just after unloading in both the flocking density groups in both seasons, which then declined to basal values ($P < 0.05$) with subsequent hours after post-transportation. A similar trend was observed for goats in hot-humid season. The maximum value of glucose levels 86.31 ± 3.23 (mg/dL) was reported at unloading in the high flocking density group compared to 74.94± 3.19 (mg/dL) in the low flocking density group during the hot-humid season. The values of glucose levels remained elevated until 24 hours post-transportation. In the winter season, high-flocking density goats of group II showed higher values ($P < 0.05$) of glucose levels at unloading as compared to low-flocking density goats. The glucose levels in the hot-humid season between the two densities varied significantly ($P < 0.05$), and the highest value, 77.00±2.50 (mg/dL), was reported in low flocking density goats after unloading in group II. The glucose levels in group II in both flocking densities during winter and hot-humid seasons were significantly lower than the other two groups. In group III, lower glucose levels were observed in low flocking density during winter and hot-humid seasons compared to high flocking densities. Significant higher values of glucose levels were recorded just after unloading in both the densities in group III in both seasons, which then declined to basal values ($P < 0.05$) with subsequent hours of post-transportation. During the hot-humid season, the maximum values of glucose levels 90.75 ± 0.39 (mg/dL) were found in high flocking density goats in

group III. Analysis of Variance for glucose levels indicated a significant difference ($P < 0.05$) between seasons, density, and groups. There was a significant ($P < 0.05$) interaction between season, density, and groups.

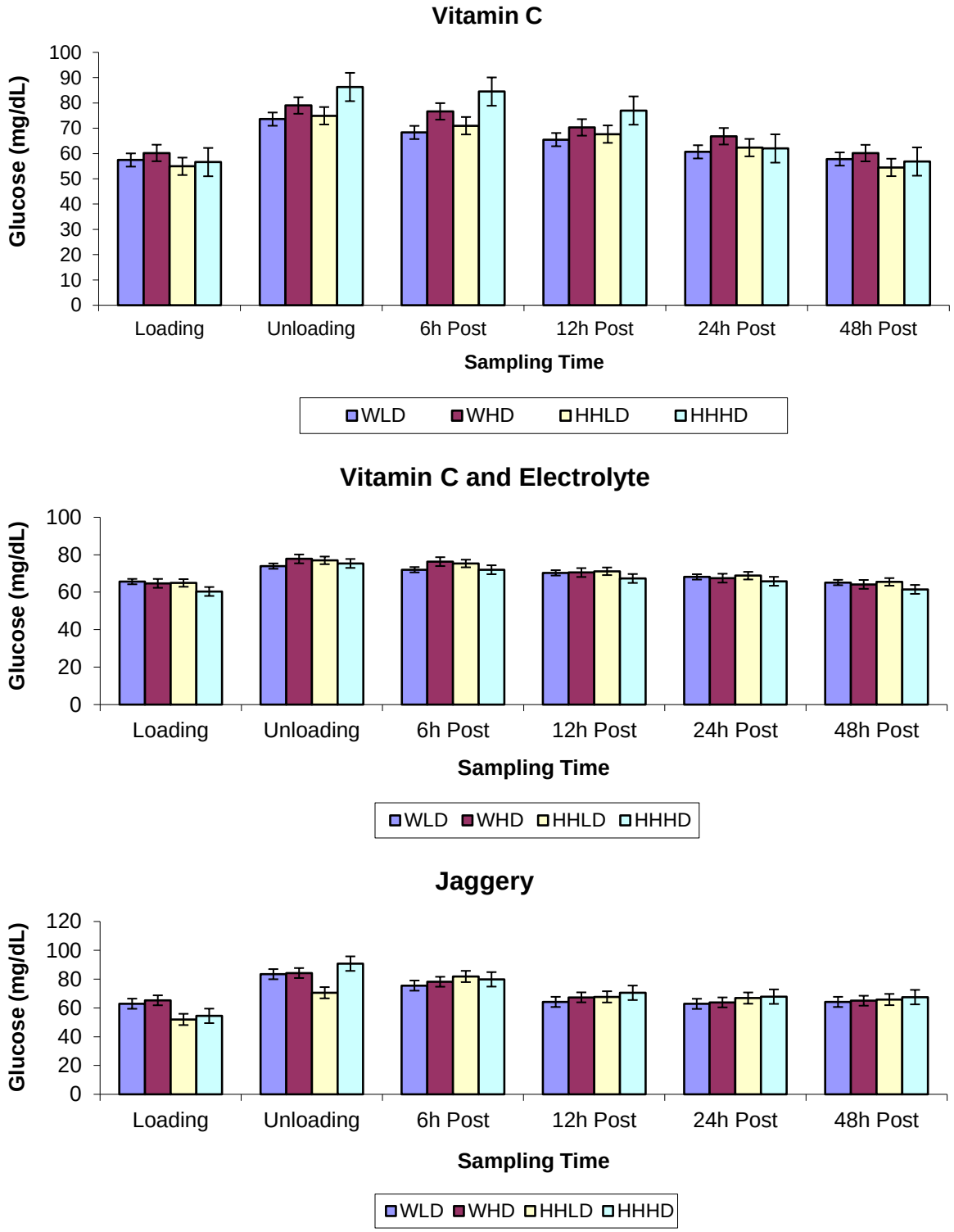

Figure 1 Average values of plasma Glucose (mg/dL) in goats transported at different flocking densities during winter and hot humid seasons.

3.2. Super Oxide Dismutase (U/mL)

The SOD activity of goats in the hot-humid season was significantly ($P < 0.05$) higher than in the winter season in both the flocking density groups. The maximum values of SOD activity were recorded just after unloading in both the flocking density groups in both seasons, which then declined to basal values ($P < 0.05$) with subsequent 6 to 12 hours after post transportation. The maximum value of SOD activity 2.38 ± 0.08 (U/mL) was reported 6 hours after unloading in the high-flocking density group

compared to 2.23 ± 0.14 (U/mL) in the low-flocking density group during the hot-humid season. The values of SOD activity remained elevated until 12 hours post-transportation. In the winter season, high-flocking density goats of group II showed no significant differences in values of SOD activity at unloading as compared to low-flocking density goats. The SOD activity in the hot-humid season between the two densities varied significantly ($P < 0.05$), and the highest value was reported in high-flocking density goats at unloading. The values of SOD activity in group II in both flocking densities during the hot-humid season were significantly higher than in the winter season. In group III, lower SOD activity values were observed in low flocking density during the winter compared to high flocking density goats. Significantly higher values of SOD activity were recorded just after unloading in both the densities in groups II and III in both seasons, which then declined to basal values ($P < 0.05$) with subsequent hours of post-transportation (Table 4). During the winter season, the maximum values of SOD activity 2.40 ± 0.02 (U/mL) were found in high flocking density goats and 2.51± 0.02 (U/mL) in low flocking density during the hot-humid season in group III.Analysis of Variance for SOD activity indicated that there was a significant difference ($P < 0.05$) between seasons, between density, and among groups (Table 6). There was a significant ($P < 0.05$) interaction between season, density, and groups.

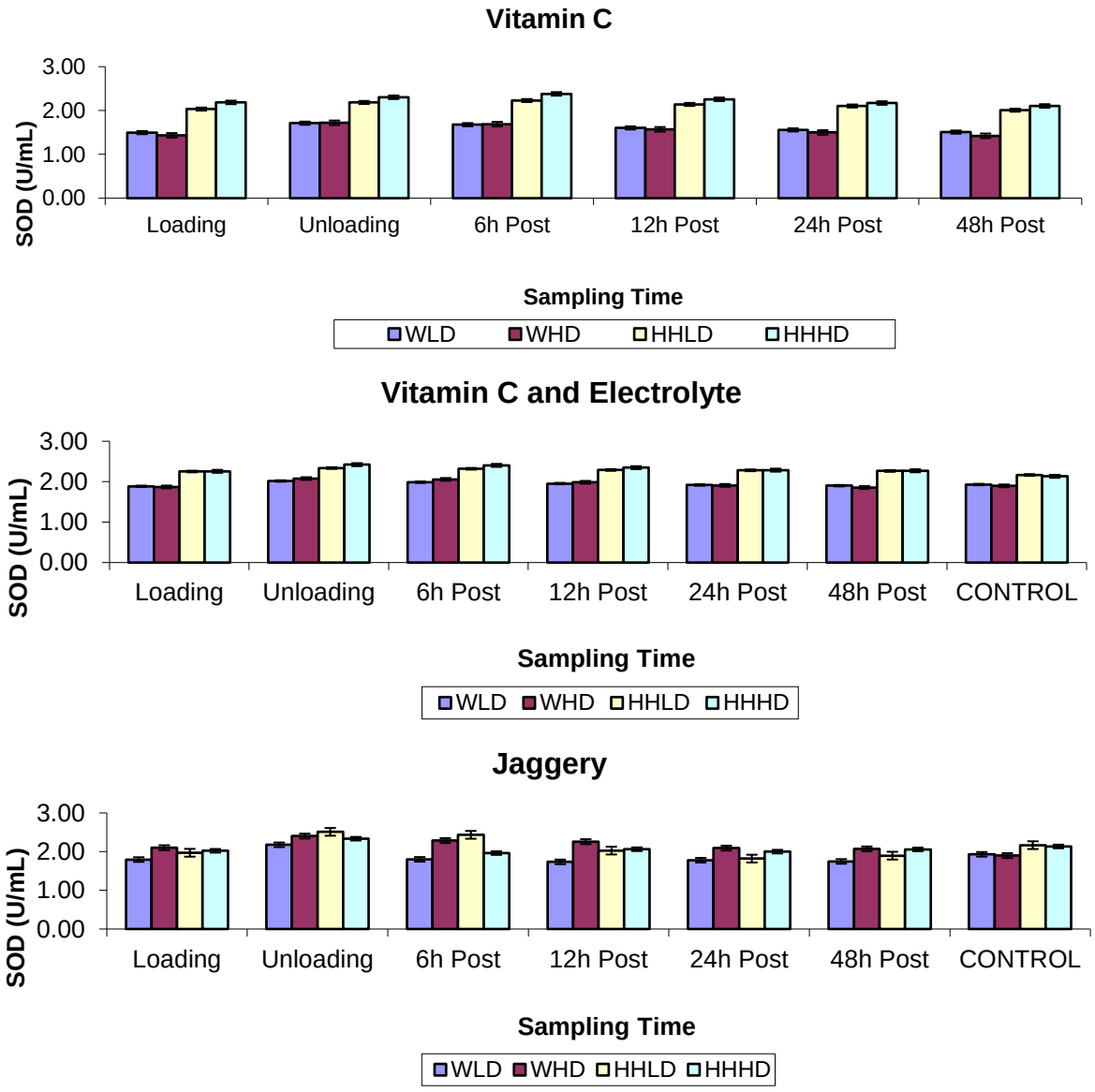

Figure 2 Average values of plasma SOD (U/mL) in goats transported at different flocking densities during winter and hot humid seasons.

3.3. Glutathione Peroxidase (GPx U/L)

In group I, in the winter season, higher values of glutathione peroxidase activity ($P < 0.05$) were observed in goats with high flocking density compared to low flocking density. The glutathione peroxidase activity of goats in the hot-humid season was significantly ($P < 0.05$) higher than in the winter season in the low-flocking density group, whereas high flocking density

group during winter showed significantly higher enzyme activity as compared to the high-flocking density goats in hot-humid season. The maximum values of glutathione peroxidase activity were recorded just after unloading in both the flocking density groups in both seasons, which continued to decline with subsequent hours after post-transportation until 24 hours. A similar trend was observed for goats in hot-humid season also. During the hot-humid season, the maximum value of glutathione peroxidase activity 231.82 ± 19.20 U/L was reported at unloading in the low flocking density group compared to 220.81± 21.90 U/L in high flocking density group I. The values of glutathione peroxidase activity remained elevated till 24 hours post-transportation. In the winter season, high-flocking density goats of group II showed higher values ($P < 0.05$) of glutathione peroxidase activity at unloading as compared to low-flocking density goats. The glutathione peroxidase activity in the hot-humid season between the two densities varied significantly ($P < 0.05$), and higher values were reported in low-flocking density goats after unloading. The values of glutathione peroxidase activity in group II in both flocking densities during the hot-humid season were significantly higher than in the winter. In group III, higher glutathione peroxidase activity was observed in low flocking densities during winter and hot-humid seasons compared to high flocking densities. Significantly higher values of glutathione peroxidase activity were recorded just after unloading in both the densities in groups II and III in both seasons, which then declined to basal values ($P < 0.05$) with subsequent hours of post-transportation. During the winter season, the maximum values of glutathione peroxidase activity 338.58 ± 14.70 U/L were found in low flocking density goats in group III. Analysis of Variance for glutathione peroxidase activity indicated a significant difference ($P < 0.05$) between seasons, density, and groups. There was a significant ($P < 0.05$) interaction between season, density, and groups (Table 5).

In a nutshell, pre-transportation values of glucose, GPx, and SOD were lower ($P < 0.05$), followed by a maximum increase in values just after loading in all the groups and both seasons. The values continued to decline with subsequent hours after post transportation until 24 hours.

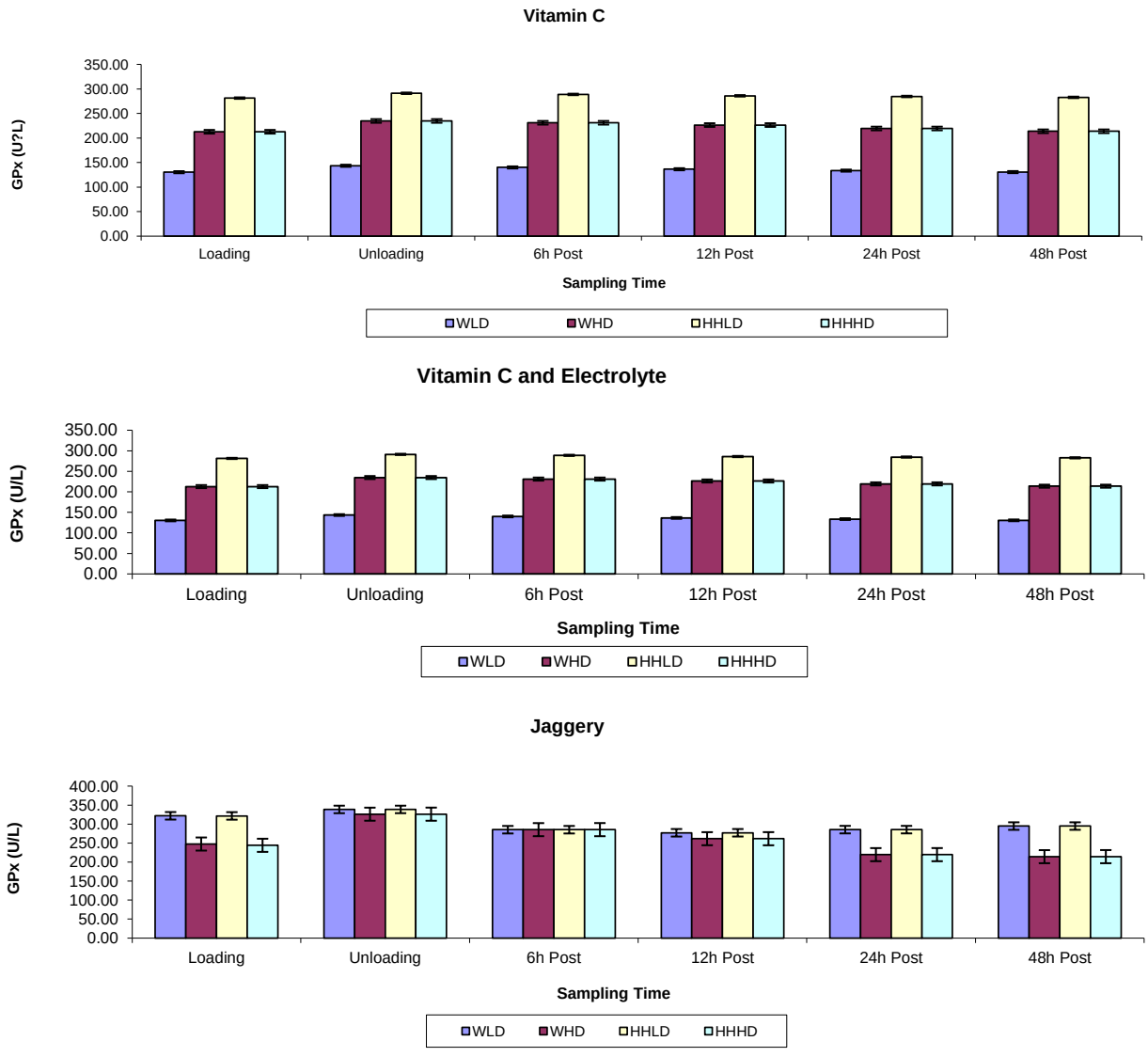

Figure 3 Average values of plasma GPx (U/L) in goats transported at different flocking densities during winter and hot humid seasons.

Table 3 Average values of plasma glucose (mg/dL) in goats transported at different flocking densities during winter and hot humid seasons.

Transportation / Groups	Winter		Hot Humid	
	Low Flocking Density	High Flocking Density	Low Flocking Density	High Flocking Density
Vitamin C (Group I)				
Before Loading	57.44 ± 1.22^{bx}	60.22± 1.09^{by}	54.94± 0.22^{bz}	56.61± 1.38 ^{bx}
After Unloading	73.61 ± 2.30^{cw}	79.00± 1.76^{cx}	74.94± 3.19^{cw}	86.31± 3.23 ^{cy}
6h Post	68.33± 3.28 ^{aw}	76.67± 3.63^{cx}	71.00± 3.67^{cy}	84.50± 0.78^{cz}
12h Post	65.50± 2.10^{aw}	70.33± 3.70^{dx}	67.67± 2.84^{aw}	77.00± 1.58^{dy}
24h Post	60.67± 1.34^{dw}	66.83± 3.64^{ax}	62.33± 1.53^{dy}	62.00± 1.67^{ey}
48h Post	57.83± 1.21^{bw}	60.17± 1.21^{cx}	54.50± 0.57^{by}	56.83± 1.23^{bw}
Vitamin C and Electrolyte Mixture (Group II)				
Before Loading	65.67± 1.93^{aw}	64.72± 2.24^{bw}	64.94± 0.77^{ew}	60.39± 0.81^{ex}
After Unloading	73.94± 2.95^{cw}	77.78± 2.11^{cx}	77.00± 2.50^{fx}	75.36± 2.35^{dw}
6h Post	72.00± 2.20^{cw}	76.33± 3.22^{cx}	75.33± 2.75^{fx}	72.00± 2.05^{cw}
12h Post	70.33± 3.70^{cw}	70.50± 2.48^{dw}	71.17± 1.95^{cw}	67.33± 1.68^{ax}
24h Post	68.17± 3.21^{aw}	67.50± 2.97^{aw}	68.83± 0.29^{aw}	65.83± 0.62^{ax}
48h Post	65.17± 0.72^{aw}	64.17± 0.69^{bw}	65.50± 3.73^{ew}	61.50± 2.93^{ex}
Jaggery Solution (Group III)				
Before Loading	62.92± 1.05^{dw}	65.28± 0.97^{bx}	52.00± 2.27^{by}	54.47± 2.64^{bz}
After Unloading	83.44± 0.57^{ew}	84.22± 0.73^{ew}	70.54± 0.48^{cx}	90.75± 0.39^{fy}
6h Post	75.50± 3.01^{cw}	78.17± 1.85^{cx}	81.83± 0.58^{gy}	79.83± 0.49^{dx}
12h Post	64.17± 1.46^{aw}	67.33± 0.87^{ax}	67.67± 0.93^{ax}	70.50± 0.71^{cy}
24h Post	62.83± 1.35^{dw}	63.83± 1.19^{bw}	66.83± 0.58^{ax}	67.83± 0.70^{ax}
48h Post	64.17± 1.19^{aw}	65.00± 2.24^{aw}	65.83± 0.58^{aw}	67.50± 0.44^{ax}

Between rows a, b, c and d differed significantly ($P < 0.05$); Between columns w, x, y and z differed significantly ($P < 0.05$).

Table 4 Average values of plasma superoxide dismutase (U/mL) in goats transported at different flocking densities during winter and hot humid seasons.

Transportation/Groups	Winter		Hot Humid	
	Low Flocking Density	High Flocking Density	Low Flocking Density	High Flocking Density
Vitamin C (Group I)				
Before Loading	1.50±0.03^{ax}	1.43±0.04^{aw}	2.03±0.05^{by}	2.18±0.06^{az}
After Unloading	1.71±0.06^{bw}	1.72±0.09^{dw}	2.18±0.06^{bx}	2.30±0.05^{by}
6h Post	1.68±0.07^{bw}	1.69±0.04^{dw}	2.23±0.14^{bx}	2.38±0.08^{cy}
12h Post	1.60±0.06^{aw}	1.57±0.05^{cw}	2.14±0.11^{bx}	2.26±0.06^{by}
24h Post	1.56±0.05^{ax}	1.50±0.01^{bw}	2.10±0.07^{by}	2.17±0.03^{ay}
48h Post	1.51±0.05^{ax}	1.42±0.01^{aw}	2.01±0.01^{by}	2.10±0.02^{az}
Vitamin C and Electrolyte Mixture (Group II)				
Before Loading	1.89±0.08^{cw}	1.87±0.09^{ew}	2.25±0.01^{bx}	2.26±0.04^{bx}
After Unloading	2.02±0.13^{cw}	2.08±0.11^{ew}	2.34±0.03^{cx}	2.42±0.07^{cy}
6h Post	1.99±0.09^{cw}	2.06±0.07^{ew}	2.32±0.03^{cx}	2.40±0.02^{cy}
12h Post	1.95±0.09^{cw}	1.99±0.09^{ew}	2.29±0.04^{bx}	2.35±0.02^{by}
24h Post	1.92±0.09^{cw}	1.91±0.09^{ew}	2.28±0.02^{bx}	2.29±0.02^{bx}
48h Post	1.90±0.10^{cw}	1.86±0.10^{ew}	2.26±0.03^{bx}	2.27±0.02^{bx}
Jaggery Solution (Group III)				
Before Loading	1.79±0.10^{bw}	2.10±0.10^{ey}	1.97±0.08^{bx}	2.02±0.06^{ax}
After Unloading	2.18±0.08^{dw}	2.40±0.02^{gy}	2.51±0.12^{ez}	2.33±0.12^{bx}
6h Post	1.80±0.08^{bw}	2.29±0.09^{fy}	2.43±0.06^{dz}	1.96±0.15^{ax}
12h Post	1.73±0.10^{bw}	2.26±0.10^{fy}	2.03±0.08^{bx}	2.06±0.08^{ax}
24h Post	1.78±0.11^{bw}	2.09±0.10^{ex}	1.82±0.12^{aw}	2.00±0.13^{ax}
48h Post	1.75±0.10^{bw}	2.07±0.08^{ey}	1.90±0.11^{ax}	2.06±0.05^{ay}

Between rows a, b, c and d differed significantly ($P < 0.05$); Between columns w, x, y and z differed significantly ($P < 0.05$).

Table 5 Average values of plasma glutathione peroxidase (U/L) in goats transported at different flocking densities during winter and hot humid seasons.

Transportation/Groups	Winter		Hot Humid	
	Low Flocking Density	High Flocking Density	Low Flocking Density	High Flocking Density
Vitamin C (Group I)				
Before Loading	163.09 ± 17.02^{bw}	199.92±21.80^{bx}	210.88±22.97^{bx}	189.55±10.02^{bx}
After Unloading	181.31 ±13.82^{cw}	231.88±12.63^{cx}	231.82±19.20^{cx}	220.81±21.90^{cx}
6h Post	180.30± 13.60^{cw}	228.81±9.51^{cx}	228.83±5.81^{cx}	218.95±13.94^{cx}
12h Post	172.15±15.33^{bw}	217.33±16.23^{bx}	223.1±20.27^{cx}	213.60±7.48^{cx}
24h Post	167.47±10.88^{bw}	210.47±14.28^{bx}	217.17±6.78^{bx}	200.50±4.53^{dx}
48h Post	163.58±7.09^{bw}	200.08±5.06^{bx}	211.67±3.21^{bx}	191.63±21.18^{dx}
Vitamin C and Electrolyte Mixture (Group II)				
Before Loading	130.29±7.55^{dw}	212.70±6.73^{bx}	281.29±22.65^{ay}	212.70±6.73^{dx}
After Unloading	143.40±20.52^{ew}	234.71±19.97^{cx}	291.15±23.31^{ay}	234.71±19.97^{ex}
6h Post	140.00±4.83^{dw}	231.00±4.89^{cx}	288.79±8.68^{ay}	231.00±4.89^{ex}
12h Post	136.33±4.11^{dw}	226.33±3.41^{cx}	285.91±11.38^{ay}	226.33±3.41^{ex}
24h Post	133.50±4.07^{dw}	219.33±1.79^{bx}	284.60±7.41^{ay}	219.33±1.79^{dx}
48h Post	130.50±4.68^{dw}	213.67±4.19^{bx}	282.74±12.15^{ay}	213.67±4.19^{dx}
Jaggery Solution (Group III)				
Before Loading	321.79±16.59^{fw}	247.55±16.39^{dw}	323.58±16.61^{ex}	247.27±11.56^{ew}
After Unloading	338.58±14.70^{fw}	326.07±7.92^{ew}	339.48±11.47^{ew}	322.07±8.12^{aw}
6h Post	285.23±8.15^{aw}	285.42±9.41^{aw}	281.13±7.05^{aw}	288.42±7.21^{fw}
12h Post	277.14 ± 5.85^{aw}	261.56±17.11^{dw}	279.10±4.65^{dw}	259.34±13.01^{fw}
24h Post	285.44±7.84^{ax}	219.63±14.98^{bw}	283.34±6.74^{ax}	222.43±11.88^{dw}
48h Post	294.83±6.82^{ax}	214.35±14.31^{bw}	297.53±7.42^{ax}	213.31±14.29^{dw}

Between rows a, b, c and d differed significantly ($P < 0.05$); Between columns w, x, y and z differed significantly ($P < 0.05$).

4. Discussion

The elevation of plasma glucose is preceded by increased plasma cortisol concentrations (Sanhouri et al 1992). In stressful conditions, plasma glucocorticoids are increased, and glucose synthesis is accelerated (Kent and Ewbank 1983; Kent and Ewbank 1986). At the same time, catecholamines secreted from the sympathetic nerve endings and adrenal medulla induces excessive glycogenolysis in the liver and muscle owing to the stimulation of phosphorylase. As a result of the absence of glucose-6-phosphatase, glycogenolysis ensues with the formation of glucose in the liver leading to an increase in blood glucose, which is responsible for increasing plasma glucose concentrations (Murray et al 1990). The rise in plasma glucose during the post-transportation period may be due to an increase in glycogenolysis, stimulated by increased secretions of catecholamine and glucocorticoid hormones, which are under the control of the sympathetic nervous system.

Similar to the current result, Ali et al (2006) found elevated plasma glucose concentration after transport in sheep and explained this result by the secondary effect of hypercortisolemia and increased glucose production from the liver, reflecting increased sympathoadrenal activity due to stress. As with the current outcome, Ali et al (2006) found a high plasma glucose concentration following sheep carriage and explained this outcome through the secondary effect of hypercortisolemia and decreased hepatic glucose production due to increased sympathoadrenal stress. An increase in plasma glucose levels as a response to transportation stress was also reported for lambs (Ekiz et al 2011) and goats (Kannan et al 2000 and 2003; Kumar 2014). During winter and hot-humid season, glucose concentration in transported groups also increased;this can be because these seasons were stressful to cross-bred goats taken in our study. The lower glucose level increase in vitamin C and electrolyte-supplemented groups compared to other groups can be because exogenous supplementation of vitamin C and electrolytes either eliminated or reduced the need for endogenous synthesis of ascorbate. The findings of the present study are also in agreement with Minka et al (2010), Averos et al (2008), Ponter et al (2003), Zulkifli et al (2001), Rajion et al (2001), Kannan et al (2000), Knowles et al (1999), Nwe et al (1996), Sanhouri et al (1992), and Kent and Ewbank (1983 and 1986).

In the present study, the up-regulation of SOD and GPx activity indicated that the goats experienced stress. An increase in SOD activity during the post-transport period was in agreement with previous studies where various stressors have been reported to increase SOD activity (Sun et al 1999; Lata et al 2004). The increased activity of superoxide dismutase in goats could be attributed to the physiological up-regulation of this enzyme in an attempt to diminish the superoxide radical challenge. Various stressors increase lipid peroxidation levels and SOD activity (Gaal et al 1993; Lata et al 2004). The higher SOD activity attributed to elevated temperatures during the summer months has been reported in cattle (Bernabucci et al 2002).

Table 6 Analysis of variance for biochemical parameters in transported goats.

Source of variation	df	Mean Sum Squares			
		Glucose	GPX	SOD	F crit.
Between Seasons	1	3.37**	306174.10**	33.65**	3.85
Between Flocking Densities	3	609.61**	222110.93**	12.03**	2.61
Between Treatments	17	3529.63**	113999.30**	1.48**	1.63
Season x Treatments	17	281.23*	20008.66*	0.86*	1.63
Densities x treatments	51	258.25*	19847.58*	0.47*	1.36
With in	1206	73.60	2594.27	0.12	
Total	1295	-	-	-	

$P < 0.05$* $P <$ 0.01**

Further post-transportation decreases in plasma SOD levels may be attributed to the increased consumption of SOD to overcome the effects of transportation. Similarly, a significant reduction in the plasma SOD activity compared to baseline values was also reported in horses transported for 12 hrs (Onmaz et al 2011), beef cattle (Celi et al 2010), and goats (Kannan et al 2007). The significant increase in GSH-Px activity might be an indirect, compensatory response of cells to increased oxidant challenge due to stressful stimuli of transportation under different temperatures. In humans, GSH-Px activity increases after long-term exercise training regimens, a response that may be an adaptive mechanism against physical stressors (Tessier et al 1995; Tauler et al 2006). From the variations in the parameters estimated, goats of the vitamin C + electrolyte group, i.e., group II showed lesser deviations from the normal values compared to other groups, indicating that a combination of vitamin C and electrolyte was more beneficial in reducing the transportation effect.

5. Conclusions

Animal transport induces physiological changes caused by handling, loading, and actual transportation. Supplementation of vitamin C, vitamin C + electrolyte, and jaggery helped to reduce individual transport stress. Still, the combination of vitamin C + electrolytes proved to be more effective in alleviating transport stress in goats.

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Ethical Considerations

Animal experimentation has been carried out following the regulations developed and authorized by the Institutional Animal Ethics Committee (IAEC) by the Livestock Research Council (LRC), National Dairy Research Institute (NDRI), India.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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References

Adenkola AY, Ayo JO, Sackey AKB, Adelaiye AB (2009) Haematological changes in pigs administered with ascorbic acid and transported by road for four hours during the harmattan season.J. Cell Anim. Biol. 3:021-028.

Al-Badwi MA, Mohamed HE, Alhaidary AA, Al-Hassan MJ (2012) Plasma and salivary cortisol levels in transportation-stressed Aardigoats.Journal of Animal & Plant Sciences 13:1731-1735.

Ali BH, Al-Qarawi AA, Mousa HM (2006) Stress associated with road transportation in desert sheep and goats, and the effect of pretreatment with xylazine or sodium betaine. Research veterinary science 80:343–348.

Averos A, Martin S, Riu M, Serratosa J, Gosalvez LF (2008) Stress response of extensively reared young bulls being transported to growing-finishing farm under Spanish summer commercial conditions. Livest. Sci. 119:174–182.

Ayo JO, Oladele SB (1996) Road transport stress in food animals in Nigeria. A Review. Nig. Vet. J. (Special Edition) 1:49-57.

Bernabucci U, Ronchi B, Lacetera N, Nardone A (2002) Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. Journal of dairy science 85:2173-2179.

Broom DM (2000) Welfare assessment and welfare problem areas during handling and transport. In: Grandin, T. (ed.), Livestock Handling and Transport, 43–62. CABI Publishing, Wallingford, UK.

Broom DM (2003) Causes of poor welfare in large animal during transport. Vet. Res. Comm. 27:515-518.

Burns RJ, Aspeslagh R (2014) Three decades of peace education around the world: An anthology. Routledge.

Celi P, Trana AD, Claps S (2010) Effects of plane of nutrition on oxidative stress in goats during the peripartum period. Vet. J. 184:95–99.

Ekiz OO, Urel M, Guner H, Mizrak AK, Dˆana A (2011) Reversible electrical reduction and oxidation of graphene oxide. Acs Nano 5:2475-2482.

Fazio E, Ferlazzo A (2003) Evaluation of stress during transport. Vet.Res. Commun. 27:519–524.

Flohe L, Gunzler WA, Schock HH (1973) Glutathione peroxidase: a selenoenzyme. FEBS Lett. 32:132–134.

Gaal T, Mezes M, Miskucza O, Ribiczey-Szabo P (1993) Effect of fasting on blood lipid peroxidation parameters of sheep. Research in veterinary science 55: 104-107.

Galipalli S, Gadiyaram KM, Kouakou B, Terril TH, Kannan G (2004) Physiological response of preslughter transportation stress in Tasco-supplemented goats. South African Journal of Animal Science 34:198-200.

Halliwell B, Chirico S (1993) Lipid peroxidation: its mechanism, measurement, and significance. Am. J. Clin. Nut. 57:715S–725S.

Kannan G, Saker KE, Terrill TH, Kouakou B, Galipalli S, Gelaye S (2007) Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. Small Ruminant Res. 73:221-227.

Kannan G, Terrill TH, Konakou B, Gelaye S (2003) Endocrine, blood metabolite and meat quality changes in goats as influenced by short term preslaughter stress. J. Anim. Sci. 81:4499-1507.

Kannan G, Terrill TH, Konakou B, Gazal OS, Gelaye S, Amoah EA, Samake S (2000) Transportation of goats: Effects on physiological stress responses and live weight loss. J. Anim. Sci. 78:1450-1457.

Kent JK, Ewbank R (1983) The effect of road transportation on the blood constituents and behavior of calves. In Six months old. Br. Vet. J. 139:228-235.

Kent JK, Ewbank R (1986) The effect of road transportation on the blood constituents and behavior of calves. II. One to three weeks old. Br. Vet. J. 142:131-140.

Khasmi ELM, Bargaâ R, Riad F, Safwate A, El Hassane T, Farh M, El Abbadi N, Abouhafs R, Faye B (2013) Meat levels of 25-hydroxyvitamin D3 in Moroccan one-humped dromedary camels (Camelus dromedarius). Emirates Journal of Food and Agriculture 25:267.

Knowles J, Corne D (1999) The pareto archived evolution strategy: A new baseline algorithm for pareto multiobjectiveoptimisation. In Evolutionary Computation, 1999. CEC 99. Proceedings of the 1999 Congress on (Vol. 1).

Knowles TG, Warriss PD, Brown SN, Edwards JE (1999) Effects on cattle transportation by road for upto 31 hours. Veterinary Record 145:575-582.

Kumar M, Jindal R, Nayyar S, Singla M (2010) Physiological and biochemical responses in beetal goats during summer season. Indian Journal of Small Ruminants, 16(1), 255-257.

Kumar BR, Muralidharan MR, Ramesh V, Arunachalam S, Sivakumar T (2003) Effects of transport stress on blood profile in sheep. Ind. Vet. J. 80, 511-514.

Kumar K (2014) Effect of transportation stress on physiological performance and immune Function of The Goats. Thesis submitted to ICAR- NDRI, Karnal.

Lata JC, Degrange V, Raynaud X, Maron PA, Lensi R, Abbadie L (2004) Grass populations control nitrification in savanna soils. Functional Ecology 18:605-611.

Lopez O, Marco I, Montane J, Lavin S (2006) Transport stress inSouthern chamois (Rupicapra pyrenaica) and its modulation by acepromazine. Vet. J. 1720:347-355.

McDowell RE, Hooven NW, Camoens JK (1976) Effects of climate on performance of Holsteins in first lactation. J. Dairy Sci. 59:965–973.

Miller JK, Brzezinska-Slebodzinska E, Madsen FC (1993) Oxidative stress, antioxidants, and animal function. J. Dairy Sci. 76:2812–2823.

Minka NS, Ayo JO (2007) Physiological responses of transported goats treated with ascorbic acid during the hot-dry season. Animal Science Journal 78:164–172.

Minka NS, Ayo JO (2007a) Effects of loading behaviour and road transportation stress on traumatic injuries in cattle transported by road during hot- dry season. Livestock Science 107:91-95.

Minka NS, Ayo JO (2010) Behavioral and rectal temperature responses of Black Harco pullets administered vitamins C and E and transported by road during the hot-dry season. Journal of Veterinary Behavior: Clinical Applications and Research 5:134-144.

Minka NS, Ayo JO (2007) Effects of loading behaviour and road transport stress on traumatic injuries in cattle transported by road during the hot-dry season. Livestock Science 107:91-95.

Minka NS, Ayo JO (2007b) Physiological responses of transported goats treated with ascorbic acid during the hot dry season. Animal Science Journal 78:162-172.

Minka NS, Ayo JO (2008) Haematology and behaviour of pullets transported by road and administered with ascorbic acid during the hot dry season. Research in Veterinary Science 85:389–393

Minka NS, Ayo JO (2010) Physiological responses of Erythrocytes of goats to Transportation and Mondulatory Role of ascorbic acid. J.Vet. Med. Sci. 72:875-881.

Minka NS, Ayo JO (2010) Serum biochemical activities and muscular soreness in transported goats administered with ascorbic acid during the hot-dry season. European Journal Translational Myology. Basic Applied Myology 1:193-203.

Miranda-de la Lama, GC, Monge P, Villarroel M, Olleta JL, García-Belenguer S, María, GA (2011) Effects of road type during transport on lamb welfare and meat quality in dry hot climates. Tropical Animal Health and Production 43:915-922.

Mstl E, Palme R (2002) Hormones as indicator of stress. Domest. Anim. Endocrinol. 23:67-74.

Murray RK, Granner DK, Mayes PA, Rodwell VW (1990) Gluconeogenesis and Control of the Blood Glucose: Hormones of the Adrenal Cortex and Adrenal Medulla. Harper's Biochem- istry. 12th edn.

Nazifi S, Razavi SM, Esmailnejad Z, Gheisari H (2009) Study on acute phase proteins (haptoglobin, serum amyloid A, fibrinogen, and ceruloplasmin) changes and their diagnostic values in bovine tropical theileriosis. Parasitology research 105:41-46.

Nwe TM, Itori E, Manda, M, Watanabe, S (1996) Significance of catecholamines and cortisol levels in blood during transportation stress in goats. Small Rum. Res. 20:129-135.

Onmaz AC, Van den Hoven, R, Gunes V, Cinar M, Kucuk O (2011) Oxidative stress in horses after a 12-hours transport period. Recl. Med. Vet. 162:213-217.

Polycarp M, Dauna Y, Musa S, Ja'afaru J, Ibrahim AK (2015) Analysis of Producer Price of Rice in Nigeria. The International Journal of Science and Technoledge 3:90.

Ponter CD, Roussel S, Tessier J, Sauvant D, Ficheux C, Boissy A (2003) Physiological effects of repeated transport in pregnant goats and their offspring. Anim.Res. 52:553-566.

Rajion MA, Soat IM, Zulkifli I, Goh YM (2001) The effects of road transport on some physiological stress measures in goats. Asian- Australian Journal of Animal Science 9:1250-1262.

Sanhouri AA, Jones RS, Dobson H (1992) Effect of xylazine on the stress response to transport in male goats. Br. Vet. J. 148:119-128.

Singh D (2013) The brand personality component of brand goodwill: some antecedents and consequences. Brand equity & advertising: Advertising's role in building strong brands, p.83.

Sun Z, Cade JR (1999) A peptide found in schizophrenia and autism causes behavioral changes in rats. Autism 3:85-95.

Tadich N, Gallo H, Bustamante H, Schwerter M, Van Schaik G (2005) Effects of transport and lairage time on some blood constituents of Friesian-Cross steers in Chile. Livest. Prod. Sci. 93:223-233.

Tarrant PV, Kenny FJ, Harrington D, Murphy M (1992) Long distance transportation of steers to slaughter: effect of stocking density on physiology, behaviour and carcass quality. Livestock Production Science 30:223-238.

Tauler P, Sureda A, Cases N, Aguiló A, Rodríguez-Marroyo JA, Villa G, Tur JA, Pons A (2006) Increased lymphocyte antioxidant defences in response to exhaustive exercise do not prevent oxidative damage. The Journal of nutritional biochemistry 17:665-671.

Teke B, Ekiz B, Akdag F, Ugurlu M, Ciftci G.,Senturk B (2014) Effects of stocking density of lambs on biochemical stress parameters and meat quality related to commercial transportation . Ann. Anim. Sci. 14:611–621.

Tessier A, Turner DR eds (1995) Metal speciation and bioavailability in aquatic systems (Vol. 3).

Zulkifli I, Che Norma MT, Chong CH, Loh TC (2001) The effects of crating and road transportation on stress and fear responses of broiler chickens treated with ascorbic acid. Arch. Geflugelk. 65:33–37.

Zulkifli I, Norbaiyah B, Cheah YW, Soleiman AF, Sazili AQ, Rajion MA, Goh YM (2010) Physiological Responses in Goats Subjected to Road Transportation under the Hot, Humid Tropical Conditions. Int. J. Ag. & Bio. 12:840–844.