Biological Rhythm Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/nbrr20

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BIOLOGICAL RHYTHM RESEARCH
2020, VOL. 51, NO. 5, 697–707
https://doi.org/10.1080/09291016.2018.1557830

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ARTICLE

Seasonal variation of stress response parameters due to induced transportation stress in goats supplemented with vitamin C and jaggery under tropical Indian climatic conditions

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ABSTRACT
The study was conducted to investigate the seasonal variation of stress response parameters due to induced transportation stress in goats during different seasons, i.e. winter, hot dry and hot humid, and to find out a novel approach to minimize the transportation and seasonal effect on goats by supplementing them with vitamin C and jaggery before transportation. Fifty goats were selected and divided into five groups, with 10 animals in each group during each season. Group 1 goats served as negative control and were neither supplemented nor transported, whereas goats in groups 2 to group 5 were transported for 10 h on the day of transportation. Group 2 goats were not supplemented; group 3 goats were supplemented with vitamin C @ 180 mg/kg bd. wt/day; group 4 goats were supplemented with jaggery @ 200g/day/animal; group 5 was supplemented with both vitamin C and jaggery for 5 days before transportation. Transportation caused stress in goats during all the seasons as represented by changes in different physiological parameters and body weight shrinkages; however, hot-humid and hot-dry seasons were more critical for reducing the physiological performance of transported goats as compared to winter season. Supplementation of vitamin C and jaggery reduced the transportation stress with variable effects on stress response parameters in goats during different seasons.

ARTICLE HISTORY
Received 12 November 2018
Accepted 25 November 2018

KEYWORDS
Goats; jaggery; season; physiology; transportation

1. Introduction

Domestication of animals paralleled with human civilization itself; thus, domestic animals became an indivisible part of human civilization. Domestic animals are kept for various purposes, e.g. as food animals, companions, drought power, for animal products and by-products depending upon the type of animal. As human beings use different transportation means for going from one place to another, the choice of the transporation depends on the duration of travel, comfort level, economy, etc.. In the same way, livestock is being transported through different means, but the most common mode utilized in the world including India is road transportation. Domestic animals are transported for various reasons or

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purposes, e.g. for slaughter at slaughterhouses which are mostly located away from animal farms, for treatment of various ailments, vaccinations, for artificial insemination (AI) at AI centres, for participation in exhibitions, fairs, melas, sports competitions or seasonal tourism and for shifting animals to pastures. Transportation involves holding of animals, loading them on to a vehicle, actual transit from loading to destination and then unloading. During transportation, animals experience acute stress. Goat transportation is also fast expanding due to increased demand for goat meat and skin (Kannan et al. 2002). The majority of the studies conducted on the transportation of goats have been done in the temperate regions of the world (Rajion et al. 2001), and at the same time measures aimed at alleviating road transportation stress in goats are still limited (Galipalli et al. 2004; Minka and Ayo 2007). Important limiting factors affecting goat transported by road in tropical countries are high ambient temperature and relative humidity (RH) occurring during the hot-humid season of the year (Rajion et al. 2001; Minka and Ayo 2007). Both oral and intramuscular routes could be used in the administration of ascorbic acid in short-term transportation stress in goats (Biobaku et al. 2018). Glycerin supply to kids reduced plasma concentrations of stress indicators during pre-slaughter lairage (Matos et al. 2018). The environment conditions, especially high or low ambient temperature and RH, add to the transportation stress of animals (Mir et al. 2018b). So the current study has been carried out to assess the effect of different seasons on transportation stress in goats and also the likely alleviating effects of vitamin C and jaggery supplementation during different seasons.

2. Materials and methods

The experimental goats were taken from Livestock Research Centre of National Dairy research Institute, Karnal, Haryana, India. Fifty (50) crossbred female goats (Alpine x Beetle) were selected during all seasons. The present work was divided into three experimental trials: experiment 1 was conducted during winter (December- January), experiment 2 during hot-dry (May–June) and experiment 3 during hot-humid (July–August) season. The selected goats were apparently healthy and were dewormed before the start of the experiment. The animals were kept in separate sheds during the period of study, and feeding was done as per the standard procedure followed by the institute.

All the goats were divided into five groups randomly based on their body weight, so that the average body weight of each group had least variation from each other. Group 1 goats served as negative control (NC) and were neither supplemented nor transported, whereas goats in groups 2 to group 5 were transported for 10 h on the day of transportation. Group 2 (positive control (PC)) goats were not supplemented; group 3 (T1) goats were supplemented with vitamin C @ 180 mg/kg bd. wt/day; group 4 (T2) goats were supplemented with jaggery @ 200g/day/animal; group 5 (T3) goats were supplemented with both vitamin C and jaggery for 5 days before transportation. In order to establish the dose of vitamin C, vitamin C at various doses was fed to goats divided into different groups. Both plasma and urine vitamin C were estimated in each goat. Doses of vitamin C fed to goat's verses concentration in plasma and urine were plotted and also days of feeding vitamin C and its concentration in blood and urine. Then, after interpretation and calculations, we concluded that feeding vitamin C @ 180 mg per kg body weight/animal/day leads to a peak value of vitamin C in plasma at the fourth day of feeding and at the same time there is minimum urinary loss of vitamin C at this dose rate. Jaggery was selected based on fact that jaggery is a good source of energy, minerals and vitamins besides being in abundance in our country and is also cost-effective. A single dose of jaggery was selected based on our hypothesis that energy supplement will synergize with antioxidant (vitamin C) in alleviating transportation stress in goats.

Blood sampling was done 5 days before transportation, at the time of loading on to a vehicle, at unloading from a vehicle after transportation and then 6 h, 12 h, 1 day and 5 days post-transportation. For blood gas analysis and haematology, blood was taken in 1 ml syringes, and for other parameters, blood was collected in Ethylenediaminetetraacetic acid (EDTA) vacutainers to get plasma. Blood gas analysis and haematology were done immediately from fresh blood samples, whereas plasma was stored at −20°C and was later used for estimation of glucose, creatine kinase (CK), serum amyloid A (SAA), cortisol, prolactin and minerals (sodium, potassium and phosphorous). Whole blood analysis for haematology was done by using BC-2800 Vet auto haematological analyzer using goat-specific programme. Blood gas analysis was done by using blood gas analyzer (stat profile pHox). Biochemical parameters, hormones and minerals were estimated using commercially available kits. Skin temperature was measured by using infrared Tele-thermometer (Raytek, Model Rayngeer ST2L, M/s. Surrey Scientific, Surrey, UK). Body weight was measured with the help of electronic weighing balance in early morning before feeding on each day of sampling. Data analysis was carried out using Sigma plot 11. Mean values at different sampling times were compared with respective basal mean values of each group using one-way ANOVA with post-test as Dunnett's multiple comparison. Animal experimentation was performed in compliance with regulations set by the Livestock Research Centre, NDRI Karnal, and approved by the Institutional Animal Ethics Committee.

3. Results

3.1. Haematological parameters

Haemoglobin concentration increased due to transportation and remained significantly higher even up to 5 days post-transportation in PC group during the hot-dry season and in PC and T_2 groups during the hot-humid season, while in T_1 and T_3 groups haemoglobin concentration returned to normal basal level at 12 h to 2 days post-transportation. There was a significant increase in PCV at unloading in all transported groups of goats during all seasons; however, maximum increase was in PC group during all seasons. In PC groups, PCV remained significantly higher up to 2 days in hot-dry and hot-humid season, while in treatment groups PCV returned to normal value quickly, i.e. 6–12 h post-transportation. There was a significant increase in RBC concentration in all transported groups of goats, during all seasons. In PC, T_2 and T_3 groups, RBC values remained significantly higher even up to 2 days post-transportation, whereas in T_1 group Red Blood Cell (RBC) values returned to normal basal values immediately post-transportation, i.e. within 6–12 h. There was a significant increase in White Blood Cell (WBC) concentration in PC and T_2 groups during all seasons and remained significantly higher even up to 5 days post transportation during the hot-humid season. In T_1 and T_3 groups, no significant difference in WBC concentration was found before and after transportation during the winter season; however, a significant increase in WBC concentration of T_1 and T_3 groups was found during hot-dry and hot-humid season, but the normal basal levels of WBC in these groups were attained earlier as compared to PC and T_1 groups of goats. There was a significant increase in neutrophil percentage in all transported groups, during all seasons with significantly higher values at unloading and 6 h post-transportation. In PC and T_2 groups, values remained significantly higher up to 2 days post-transportation during the hot-dry and hot-humid seasons, while in T_1 and T_3 groups neutrophil percentage returned to normal basal level at 1 day post-transportation during all seasons. There was a significant decrease in lymphocyte percentage in all transported groups of goats during all seasons, with significantly lower values at unloading and 6 h post-transportation. In PC and T_2 groups, lymphocyte percentage remained significantly lower even up to 2 days during hot-dry and hot-humid seasons, while in T_1 and T_3 groups, lymphocyte percentage returned to normal basal level at 1 day post-transportation. During the winter season, N/L ratio was significantly higher post-transportation only in PC group. In PC and T_2 groups, N/L ratio remained higher up to 12 h to 2 days post-transportation during hot-dry and hot-humid seasons, while in T_1 and T_3 groups, N/L ratio returned to normal level only at 6–12 h post-transportation.

3.2. Biochemical parameters

Glucose level increased due to transportation and peaked at unloading in all transported groups of goats. In PC groups, there was 10%, 53% and 86% increase in glucose level at unloading during winter, hot-dry and hot-humid seasons, respectively. In PC and T_1 groups, glucose level returned to normal basal level 5 days post-transportation during hot-dry and hot-humid seasons. In NC groups, glucose level also increased after 10 h of feed and water restriction during hot-dry and hot-humid seasons.

There was a significant increase of creatine kinase (CK) activity due to transportation in all transported groups of goats during all seasons. During winter and hot-dry seasons, CK activity in transported goats returned to normal basal level at 5 days post-transportation, while during hot-humid season, levels were significantly higher even at 5 days post-transportation. The CK activity increased maximum during the hot-humid season, followed by hot-dry and winter seasons.

SAA increased post-transportation in all the transported groups of goats. A maximum increase was found in PC groups during all the seasons, and peak values were recorded at 12 h to 2 days post-transportation. During hot-humid season, higher level of SAA was found as compared to hot-dry and winter seasons in all groups of goats.

3.3. Hormones

There was a significant increase in cortisol concentration of PC groups at unloading during all seasons and remained significantly elevated up to 12 h post-transportation during hot-dry and hot-humid seasons. In T_1, T_2 and T_3 groups, there was either no significant increase in cortisol concentration or normal values were obtained at 6 h post-transportation.

Prolactin concentration was higher post-transportation at unloading in PC and T_2 groups during all seasons and returned to normal value 6–12 h post-transportation, while in T_1 and T_3 groups, there was no significant increase in prolactin concentration due to transportation.

3.4. Blood minerals

There was a significant decrease in sodium concentration in all transported groups of goats, during all seasons. In T_2 group, sodium level remained significantly lower up to 2–5 days post-transportation during all seasons, while in other transported groups sodium level returned to normal level 6–12 h post-transportation. During the hot-dry season, sodium level in NC group increased significantly and remained higher up to 12 h.

There was a significant decrease in potassium concentration in all transported groups of goats, during all seasons. The potassium concentration remained significantly lower at unloading, 6 h, 12 h and even at 2 days in some transported groups of animals.

There was a significant increase in phosphorous concentration in PC and T_1 groups of goats during all seasons due to transportation. In T_2 and T_3 groups, no significant difference was found in magnesium concentration before and after transportation during winter and hot-dry seasons, while in hot-humid season, a significant increase of phosphorous concentration was found in T_2 and T_3 groups also.

There was a significant decrease in magnesium concentration in PC and T_1 groups of goats, during all seasons due to transportation. During hot-dry season, magnesium concentration returned to normal basal level only after 5 days post-transportation. In T_2 and T_3 groups, no significant difference was found in magnesium concentration before and after transportation during any season.

3.5. Blood gas analysis

There was a significant decrease in pCO_2 value in all transported groups and differed significantly from normal basal values at unloading during all seasons. The maximum decrease of pCO_2 concentration was found in PC group during the hot-humid season, followed by hot-dry and winter season.

There was a significant increase in pO_2 value in all transported groups of goats, during all seasons. During winter and hot-dry seasons, pO_2 values returned to normal basal values quickly as compared to the hot-humid season.

There was no significant effect of transportation on blood pH in any of the transported groups during any season.

3.6. Physiological parameters

There was a significant increase in respiration rate in all transported groups of animals during all seasons. Respiration rate at unloading was significantly higher compared to basal values in all groups of goats; however, no significant difference was found from 6 h post-transportation. Maximum values of respiration rate were found during the hot-humid season, followed by hot-dry and winter season.

There was a significant increase in pulse rate in PC groups during all seasons. In treatment groups (T_1, T_2 and T_3), pulse rate was either not increased or returned to normal basal level immediately, i.e. within 6 h post-transportation.

Rectal temperature increased significantly due to transportation in all transported groups of goats, during all seasons. Rectal temperature returned to normal basal level immediately, i.e. within 6–12 h post-transportation in all groups of animals during all seasons.

There was a significant increase in skin temperature in all transported groups of goats, during all seasons. Skin temperature differed from basal value only at unloading during all seasons. Highest peak values of skin temperatures at unloading were observed during the hot-dry season compared to hot-humid and winter seasons.

3.7. Body weight shrinkage

There was a significant body weight shrinkage (average 2.5 kg/7% of body weight) in PC group at unloading during all seasons; however, in T_1, T_2 and T_3 groups, body weight shrinkage (average 1 kg/2.5% of body weight) was not significant at unloading during winter and hot- humid seasons. In the PC group, goats were not able to regain body weight even after 2 days post-transportation during the hot-dry season.

4. Discussion

4.1. Haematological parameters

The increase in Hb concentration in transported groups is in agreement with other workers, who also reported an increase in Hb concentration after transportation. Ambore et al. (2009) observed that transporting goat for 12 h over a distance of about 350 km had increased Hb concentration. Similarly, Minka and Ayo (2010) reported that in ascorbic acid-supplemented goats plasma Hb levels returned to normal level within 3 days post-transportation, while in control goats, they were significantly higher on the same day.

The increase of PCV due to transportation in goats can be due to dehydration, which led to hemoconcentration, and similar results have been reported by different workers. Goats transported for 12 h over a distance of about 350 km revealed a significant increase in the haematocrit (Ambore et al. 2009). Similarly, Plyaschenko and Sidorov (1987); Rajion et al. (2001) also reported higher haematocrit values in goats after transportation.

The increase in RBC concentration can be due to (1) more immature RBCs recruited into circulation to meet the increased oxygen demand; (2) release of more RBCs into circulation due to splenic contraction and (3) secondary effect of dehydration. The increase in RBC concentration due to transportation in goats is also depicted by the study of Kumar (2014) which reported that the RBC values increased after 8 h transportation and attain the pre-transportation level after 24 h post-transportation. Also, Mir et al. (2018a) reported that RBC concentration in goats increased after transportation during the hot-dry season.

The increase in neutrophil count due to transportation can be due to (1) demargination of neutrophils, (2) delayed apoptosis of neutrophils and (3) stimulation of stem cells by growing factors like granulocyte colony-stimulating factor (G-CSF). The findings are in concomitance with those of Kannan et al. (2000) who also reported an increased percentage of neutrophil and decreased lymphocytes due to transportation stress, and the N:L ratios were higher at all time periods after transportation than prior to the beginning of transportation of goats.

The decrease in lymphocyte count due to transportation can be due to (1) margination and redistribution of lymphocytes within the lymphatic system (reticuloendothelial system), (2) marked accelerated apoptosis of lymphocytes and (3) higher serum concentration of catecholamines, prolactin and cortisol which can also induce lymphocytopenia.

4.2. Biochemical parameters

4.2.1. Glucose

The increase 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. An increase in plasma glucose level as a response to transportation stress was also reported for lambs (Ekiz et al. 2011; Morán et al. 2017) and goats (Kannan et al. 2000, 2003; Kumar 2014; Mir et al. 2018a). Lower increase of glucose level in vitamin C-supplemented groups as compared to PC groups can be due to fact that exogenous supplementation of vitamin C either eliminated or reduced the need for endogenous synthesis of ascorbate, as the increase in glucose in PC group can partially be attributed to increased synthesis of ascorbate.

4.2.2. Creatine kinase

CK increases in the blood after tissue damage, poor muscular tissue reperfusion and increase in the permeability of muscle membrane. Basically, CK catalyses the reversible transfer of phosphate from ATP to creatine, and hence play a role in temporal energy buffering by maintaining an adequate ATP/ADP ratio during interruption of energy supply or during changing energy demand. The increase in CK in all transported groups of goats can be due to the above-mentioned causes.

4.2.3. Serum amyloid A

The delayed increase in SAA in all transported groups of goats can be due to the following reasons: (1) stimulus for triggering SAA synthesis may have occurred only during the last hours of transportation, i.e. ninth to tenth hour of transportation, during which goats were fatigued and more stressed; (2) steroids (e.g. cortisol) are known to have a permissive effect on hepatic SAA synthesis and thus SAA synthesis was triggered only after cortisol levels were elevated to a level to have a permissive effect on SAA synthesis. Serum concentration of serum amyloid A (75.43 ± 2.11) was significantly ($P \leq 0.05$) higher in calves transported for 4 h in open truck when compared with control calves 23.9 ± 0.56 mg/l as reported by EL-Deeb and EL-Bahr (2014).

4.3. Hormones

4.3.1. Cortisol

The increase of cortisol in transported groups of goats might be due to activation of the sympathetic nervous system. In vitamin C-supplemented groups, the cortisol level was either not elevated or returned to normal basal values immediately; this can be due to reduced synthesis or secretion of cortisol and/or breakdown of cortisol by vitamin C; however exact mechanisms are yet to be fully understood. The increase in cortisol due to transportation stress in our study is also corroborated by other workers (Kannan et al. 2000; Grigor et al. 2004; Ekiz et al. 2011; Morán et al. 2017)

4.3.2. Prolactin

The elevated prolactin due to transportation may be involved in meeting the electrolyte and water requirements during transportation stress. Increase in prolactin concentration in goats during heat stress has been reported. Sivakumar et al. (2010) reported that prolactin level in the heat stress group of goats was increased and also differed significantly from either control or treatment groups. A similar increase in prolactin levels during heat stress was also reported by Abdel-Samee et al. (1992) in goats.

4.4. Blood minerals

4.4.1. Sodium

The decrease in blood sodium concentration can be due to the following reasons: (1) inhibitory effect of cortisol on antidiuretic hormone (ADH), thus inhibiting its water retentive effect and hence dieresis and (2) cortisol increases the secretion and efficiency of atrial natriuretic peptide (ANP) due to which there is enhanced water excretion. However, some reports show that plasma concentrations of Na remain within the normal values in transported cattle, steer and calves (Atkinson 1992; Parker et al. 2003b).

4.4.2. Potassium

Lower potassium concentration post-transportation as indicated by our results is also in agreement with other authors. Parker et al. (2003b) reported that transported *Bos indicus* steers animals had lower concentrations of plasma K compared with the control animals. It is well-recognized that stressor-induced activation of the hypothalamo–pituitary–adrenal axis stimulates the secretion of cortisol, resulting in the excretion of K (Parker et al. 2003a). The hypokalemia associated with the transported group may also be the result of a lack of feed intake.

4.4.3. Phosphorus

Phosphorous concentration was not affected by transportation, indicating the role of jaggery in maintaining the phosphorous level of transported goats. Galyean et al. (1981) reported plasma phosphate ion concentration to be higher in fasted and transported animals for 32 h. However, some workers have reported no change in phosphate concentration in transported animals. Plasma concentrations of Ca, Na, Cl and P were observed to remain within the normal values in transported cattle, steer and calves (Atkinson 1992; Parker et al. 2003b).

4.4.4. Magnesium

The decrease in magnesium concentration in goats due to transportation in our study is corroborated by other workers also. Blood magnesium level of jaggery-supplemented goats was not affected due to transportation in our study, which can be attributed to the fact that jaggery is a good source of minerals especially magnesium. As jaggery was fed for 5 days before transportation, goats may have built up higher blood magnesium concentration.

4.5. Blood gas analysis

4.5.1. pCO_2

The reduction in pCO_2 in our study might be due to: (i) reduced CO_2-combining capacity with haemoglobin and (ii) excessive elimination of CO_2 by hyperventilation. The lower value of pCO2 in transported animals is also reported by Parker et al. (2003b).

4.5.2. pO_2

The increase in pO_2 in our study might be due to increased alveolar ventilation, as respiration rate increased in order to meet more oxygen demand of body under stress. The increase in pO2 value in transported calves is also reported by Steinhardt and Thielscher (2000).

4.5.3. Blood pH

The insignificant change in blood pH can be due to efficient buffering mechanism in goats. The insignificant effect on blood pH due to transportation in our study is also supported by various workers (Schaefer et al. 1988; Parker et al. 2003b; Salama et al. 2014).

4.6. Physiological parameters

The increase in physiological responses due to transportation is also reported by several authors (Das et al. 2001; Minka et al. 2009; Zulkifli et al. 2010; Kassab and Mohammed 2014; Mir et al. 2018a). The insignificant increase of rectal temperature in vitamin C-supplemented groups can be due to the fact that vitamin C and E directly alter thermal set point by decreasing prostaglandin output, especially Prostaglandin E (PGE) series, whose turnover increases during stress and which has a direct effect on the hypothalamic thermoregulatory zone. Bello et al. (2015) reported that the goats administered ascorbic acid had a consistently lower rectal temperature values, and, thus, the administration of ascorbic acid may be beneficial to goats during the early rainy season as it reduces the negative effects of thermal environmental stress on rectal temperature.

4.7. Live weigh shrinkage

The live weight shrinkage in transported goats can be partly contributed to dehydration as represented by increased PCV of transported goats and can be also contributed to increased protein catabolism due to increased cortisol. The insignificant live body weight shrinkage in vitamin C groups can be due to less muscle catabolism. Live weight shrinkage as found in our study is also reported by various workers; however, the per cent shrinkage varies with transport duration, age of animals and climatic conditions. Live weight shrinkage due to transportation is reported by several authors (Knowles and Warriss 2007; Ritter et al. 2008; Minka et al. 2009; Mir et al. 2018a). Lower body weight shrinkage in vitamin C-supplemented goats has also been reported by several authors (Minka and Ayo 2007; Mir et al. 2018a).

5. Conclusion

Transportation up to 10 h induced stress in goats during all the seasons, i.e. winter, hot-dry and hot-humid as represented by changes in different physiological parameters and body weight shrinkages. The hot-humid and hot-dry seasons were more critical for reducing the physiological performance of transported goats as compared to the winter season. Supplementation of vitamin C and jaggery aided in reducing transportation stress individually, with variable effects on stress response parameters and their combination synergistically proved promising in alleviating transportation stress in goats.

Disclosure statement

No potential conflict of interest was reported by the authors.

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