Original Research

Modulating Effect of Ascorbic Acid, Electrolyte, Jaggery on Transport-Induced Immunosuppression in Tropical Goats in Hot Humid and Winter Seasons

Deepanshu Gupta[1], Manju Ashutosh[1], Gayatri Kashyap[2]*, Ashutosh[1], Brijesh Patel[3] and Sarvesh Kumar Rai[4]

[1]Animal Physiology division, NDRI, Karnal-132001, Haryana, INDIA
[2]Division of Pathology, IVRI, Bareilly- 243122, Uttar Pradesh, INDIA
[3]LPM division, NDRI, Karnal-132001, Haryana, INDIA
[4]Project Associate, WII, Dehradun-248001, Uttara Khand, INDIA

***Corresponding author:** gkashyap78@gmail.com

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Abstract

Present study was undertaken to evaluate the seasonal effects of transportation of goats (Alpine x Beetle) at different flocking densities, supplemented with Vitamin C in group I, Vitamin C + electrolyte in group II and Jaggery in group III, 3 days before transport of animal, during winter and hot-humid seasons. The goats were selected 10-12 months old, divided into high (15) and low (10) flocking densities, transported for 8h with average speed of 25 Km/h. All the animals were kept off-feed and deprived of water during the transportation period. Blood samples were taken just before transportation, immediately after transportation, 6hrs, 12 hrs, 24 hrs and 2 days post transportation from all the three groups, and were further analysed for estimation of different haematological parameters (WBC, neutrophil, lymphocyte and NL ratio). Maximum values ($P<0.05$) of WBC, Neutrophils , NL ratio and minimum value ($P<0.05$) of lymphocytes were observed just after unloading which then returned to normal values within 6-12 hours post-transportation at low and high flocking density in both the seasons. Among the supplements provided (Vitamin C, Vitamin C + electrolyte and jaggery), Vitamin C + electrolyte combination proved more beneficial in alleviating transportation stress in the goats.

Key words: Vitamin C, Electrolyte, Flocking Density, Immunosupression, Jaggery, Transportation Stress

Abbreviation

White Blood Cells (WBC), Neutrophil Lymphocyte ratio (NLR), Hot Humid (HH), Ascorbic Acid (AA), Low Flocking Density (lfd), High Flocking Density (hfd), Livestock Research Centre (LRC)

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Introduction

Road transportation of food animals is known to be stressful, resulting in increased morbidity and mortality. Transportation stress factors such as novelty of environment, deprivation of food and water, long journey duration, overcrowding, vehicle noise and vibration, and extreme environmental conditions are known to significantly weaken the animal's body resistance to diseases by depressing cellular and humoral immunity and transportation of animals is an inevitable husbandry practice (Fazio & Ferlazzo, 2003) and there is a need for quantitative assessment of their welfare during transportation. It includes the process of handling, loading and unloading that itself represents the most stressful period compared to the journey itself (Minka and Ayo, 2008). The majority of the studies conducted on the transportation of goats are done in temperate regions of the world (Rajion *et al*., 2001) and measures aimed at alleviating road transportation stress in goats are still limited (Galipalli *et al*., 2004 and Minka and Ayo, 2007b). Besides, little or no information is available on the effect of long-term journey duration on immune status of goats. Recently, transportation of goats has expanded worldwide due to the increased demand in goat meat and skin (Minka and Ayo, 2007). High ambient temperature and relative humidity occurring during hot-dry and hot-humid seasons of the year causes increased mortality, morbidity, loss in live mass, dehydration and poor meat quality, resulting in considerable economic losses (Rajion *et al*., 2001; Minka and Ayo 2007a and b).

According to Fazio and Ferlazzo (2003), stocking density is a major factor determining the welfare of animals in transit. High stocking densities on transport vehicles have been closely associated with greater physiological stress reactions and poorer meat quality, when compared with medium and low stocking densities (Broom, 2000). Neutrophil/lymphocyte (N/L) ratio has been used as reliable welfare indices in evaluating the immune status and adaptability of animals to various stress factors (Stanger *et al.,* 2005). Ambore *et al*. (2009) reported that transportation for 12hrs in goats resulted in an increase in total leucocyte count concentration post transportation. Minka and Ayo (2009) have reported that post-transportation, the goats had higher ($P<0.05$) neutrophil count, while lymphocyte count was lower ($P<0.05$) compared to corresponding base-line values and neutrophil:lymphocyte ratio (N:L) was significantly higher after transportation, similarly various studies have been conducted (Zulkifli *et al*., 2010, Kassab and Mohammed, 2014) indicated that ascorbic acid protect membrane integrity of various blood cells of livestock during stress. Ascorbic acid has been reported to be a chain breaking antioxidant, involved in the prevention and restriction of free radical chain formation and propagation, consequently, protecting blood cells, including neutrophils and lymphocytes from oxidative damage (Powers and Jackson, 2008). Present study investigated the comparative ameliorative effects of Vitamin C, Vitamin C + electrolyte and jaggery after loading, unloading after transportation on some blood parameters in goats raised extensively in tropical country during hot humid and winter season.

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Materials and Methods

Experimental Site and Thermal Environment Conditions

Animal experimentation was performed in compliance with regulations set by the Livestock Research Council (LRC), National Dairy Research Institute (NDRI), India and approved by the Institutional Animal Ethics Committee (IAEC). The site is situated at an altitude of 250 meters above sea level and at 29 º42' N latitude and 77º 02' E longitude. The maximum temperature recorded goes up to 45º C in summer and minimum temperature 3.5º to 4º C in winter. The average rainfall is about 700 mm. The experiment was conducted in two seasons: hot humid season (September-October) and winter season (December-January).

Animals and Experimental Plan

Cross bred goats (Alpine x Beetal) of 10-12 months of age were divided into three groups obtained from LRC, NDRI Karnal. Group I, II and III consisted of 25 goats divided into high (15) and low (10) flocking densities 0.14 mt^2 (lfd) and 0.20 m^2 (hfd) per goat (The Australian Standards and Guidelines for the Welfare of animals, 2011) of 20-25 kg respectively, continuous 8 hours transport in a tractor trailer with minimum speed of 25 km/h for 3 days since 7am to 3pm. During the journey all the animals were kept off-feed and deprived of water.

Group I was fed vitamin C at a dose of 180 mg/kg.bwt/day/animal orally. Group II was fed with vitamin C + electrolyte (180 mg/kg.bwt/day/animal of vitamin C + 7 g / animal / day Electral powder) and group III was fed jaggery at dose of 200 g/animal/day orally with small amount of concentrate, 3 days before the start of 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:

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

Sampling Procedure

The animals allotted for experimentation were housed at a same place 5 days before the start of transportation trailer during which one blood sample was taken to record the basal values of all the animals. Blood samples were taken just before transportation, immediately after transportation, 6hrs, 12 hrs, 24 hrs and 2 days post transportation in heparinized vacutainer tubes and immediately placed in icepacks and brought to the laboratory. About 1 ml blood sample from each tubes were taken in microvials for determining hematological parameters in BC-2800VET auto Haematological analyzer using goat specific programme and parameters like total leukocyte count (TLC), Neutrophils, Lymphocytes were determined

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within one hour of blood collection. The chemicals used for analyzer were purchased from Mindray Medicals India Pvt. Ltd., Gurgaon (Haryana).

Environmental Parameters

These parameters indicates study were conducted in hot humid area in India. Minimum and maximum ambient temperatures, dry bulb and wet bulb temperatures were recorded with respective thermometer for microenvironment of the experimental goats inside shed and vehicle at the time of sampling and recording physiological responses. The THI was calculated from dry bulb and wet bulb temperatures using the equation-

$$THI = 0.72 \times (C_{db} + C_{wb}) + 40.6 \text{ (McDowell } et\ al.\text{, 1976)}$$

Daily relative humidity was calculated by difference of dry bulb temperature and wet bulb temperature.

Statistical Analysis

Data analysis was carried out using SAS 9 software licensed to NDRI. 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 comparison.

Result and Discussion

Meteorological data in the month of Sep-Oct and Dec-Jan THI was 77.14 and 58.74 respectively. This suggest a hot humid (HH) and winter seasons in Karnal, India. Average values of haematological parameters WBC, neutrophils, lymphocyte and NL ratio shown in (Fig. 1 to 4) obtained in all the three groups of goats at lfd and hfd, during winter and hot-humid seasons and the analysis of variance in Table 1 for WBC, neutrophil, lymphocyte and NL ratio indicated that there was a significant difference ($P<0.05$) between seasons, between density and between groups.

Table 1: Analysis of variance for blood parameters in transported goats

Source of Variation	df	Mean Sum Squares				
		WBC	Neutrophils	Lymphocyte	N/L Ratio	F crit.
Between Seasons	**1**	82.73**	135.62**	1150.42**	0.40**	3.85
Between Densities	**3**	31.16**	96.3**	542.93**	0.36**	2.61
Between Treatments	**17**	60.7**	2238.59**	2174.51**	3.92**	1.63
Season x Treatments	**17**	13.18*	44.42*	171.64*	0.16*	1.63
Densities x treatments	**51**	5.99*	31.17*	99.72*	0.09*	1.36
With in	**1206**	0.34	4.56	10.17	0.01	
Total	**1295**	-	-			

P< 0.05 P< 0.01***

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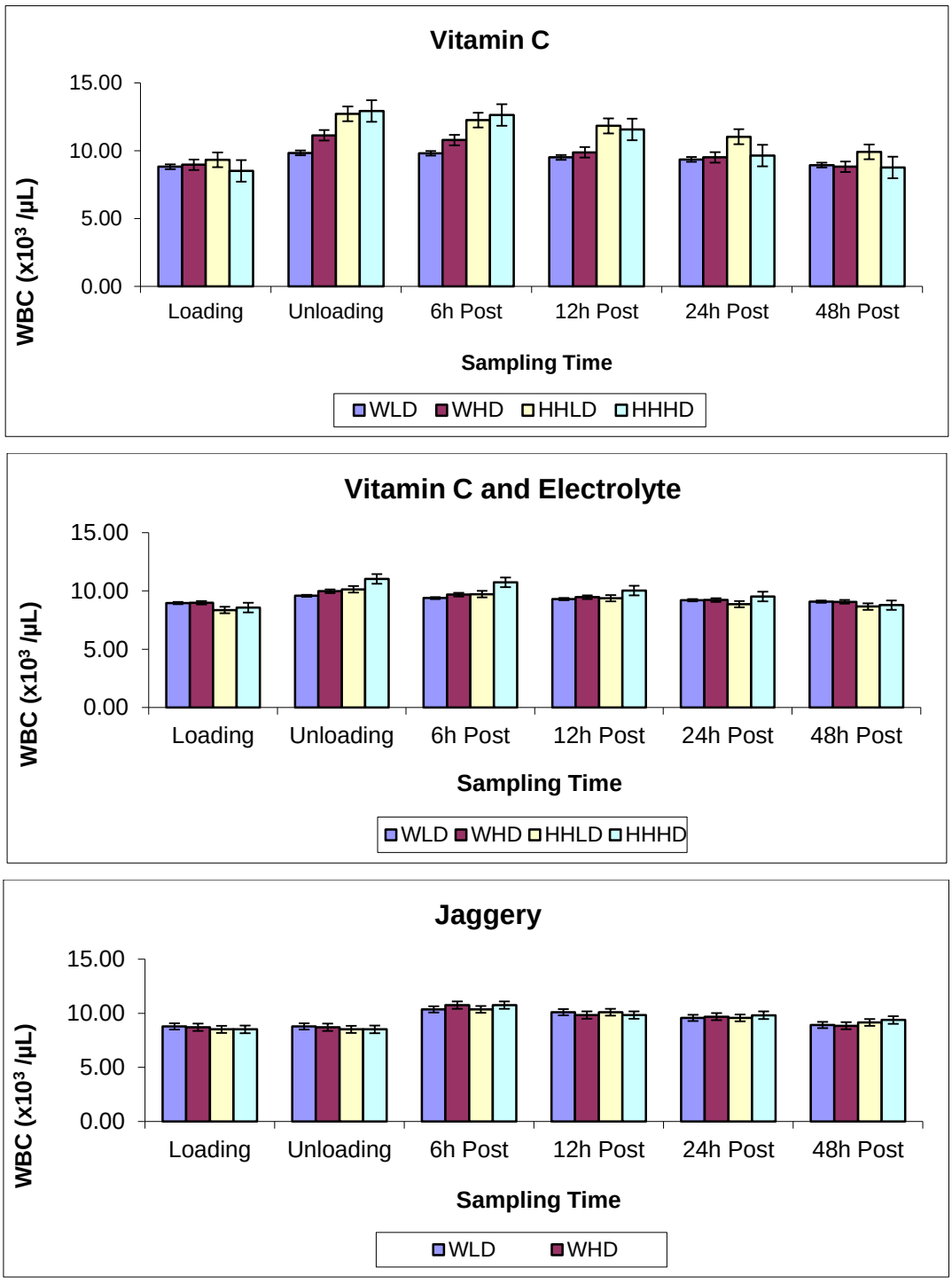

Fig. 1: Average values of wbc (x 10^3/µL) in goats transported at different flocking densities during winter and hot humid seasons

Maximum values (P<0.05) of WBC, neutrophils and NL ratio and minimum value (P<0.05) of lymphocytes were recorded just after unloading which then returned to normal values post-transportation in both the seasons and in both flocking density groups.

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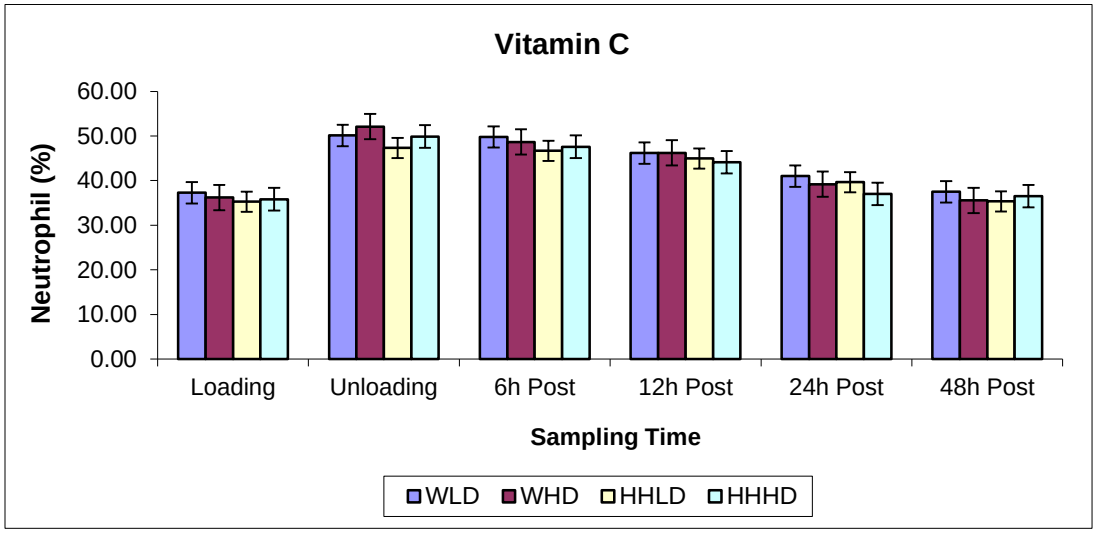

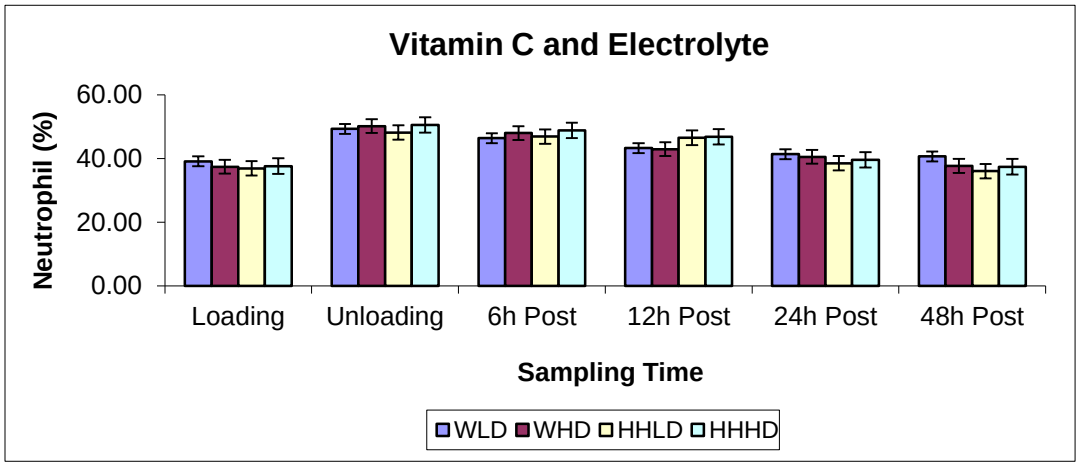

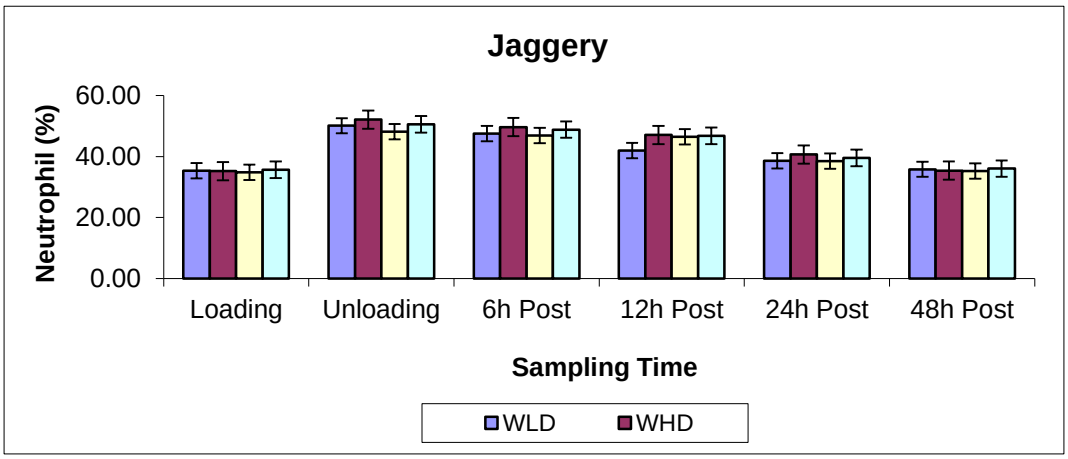

Fig. 2: Average values of neutrophils (%) in goats transported at different flocking densities during winter and hot humid seasons

In all the three groups, in both the seasons, higher values of WBC ($P<0.05$) were observed in goats with hfd in comparison to lfd goats. The values of WBCs remained significantly higher even after 24 hours post

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transportation in lfd goats during hot humid season. The group III exhibited highest WBC values as compared to group I and II in both the flocking density goats during winter and hot humid seasons.

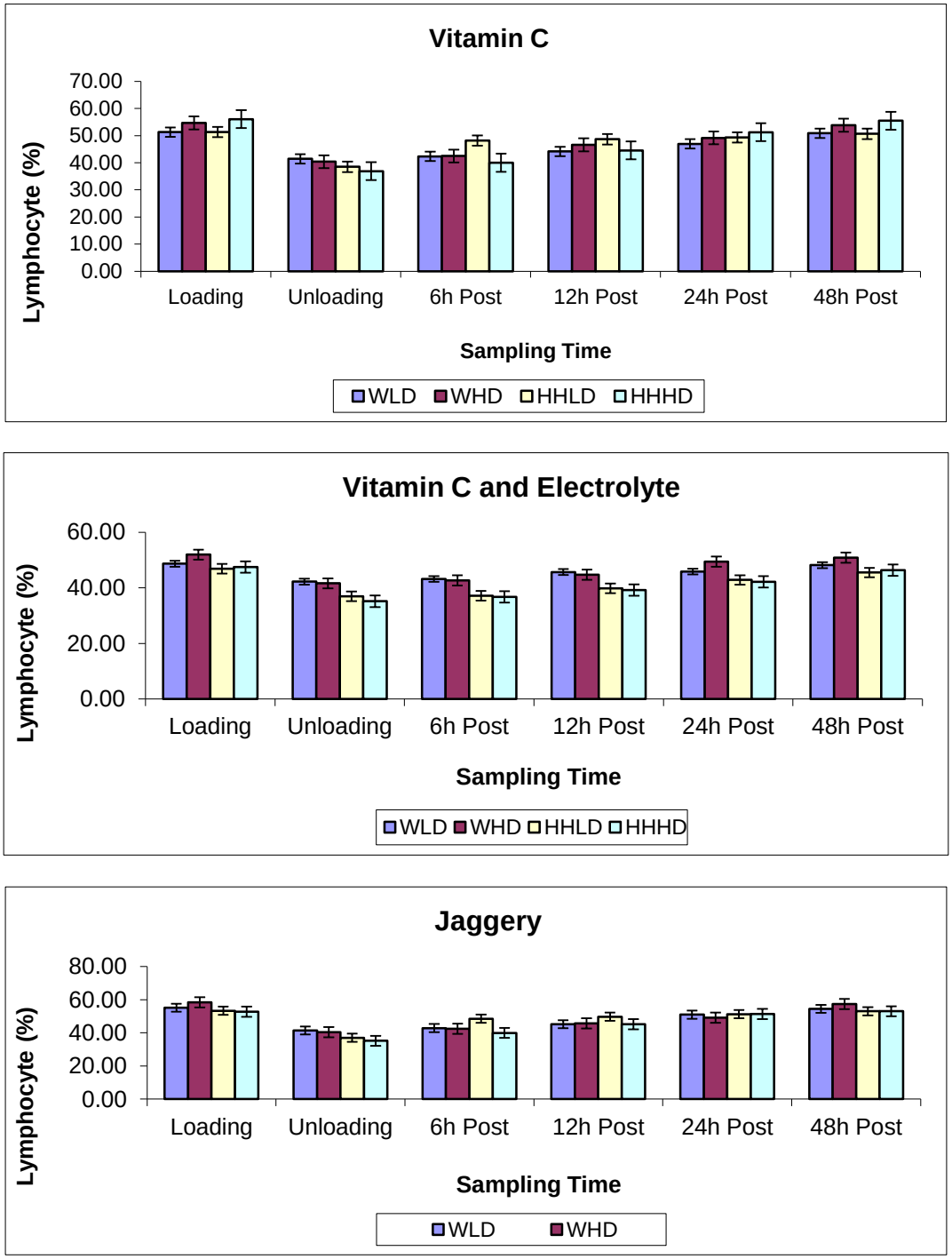

Fig. 3: Average values of lymphocytes (%) in goats transported at different flocking densities during winter and hot humid seasons

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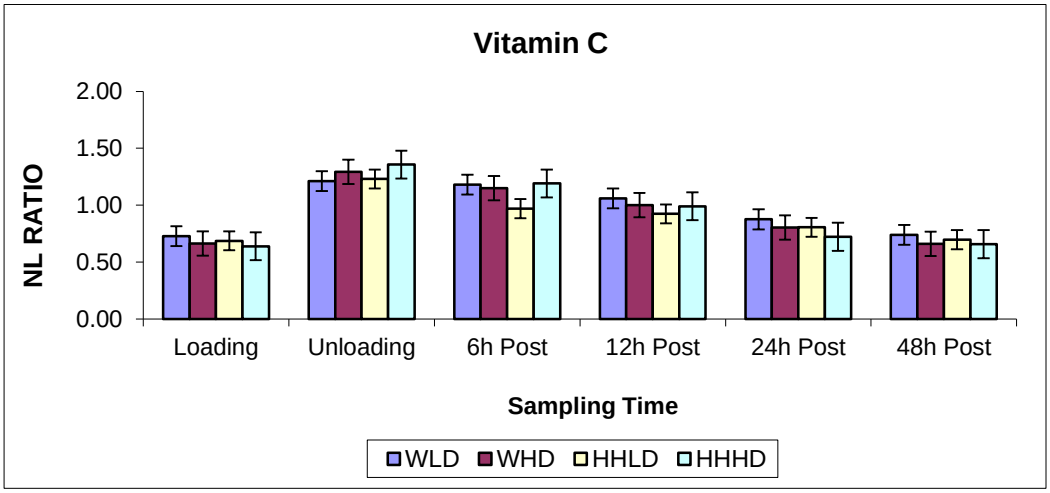

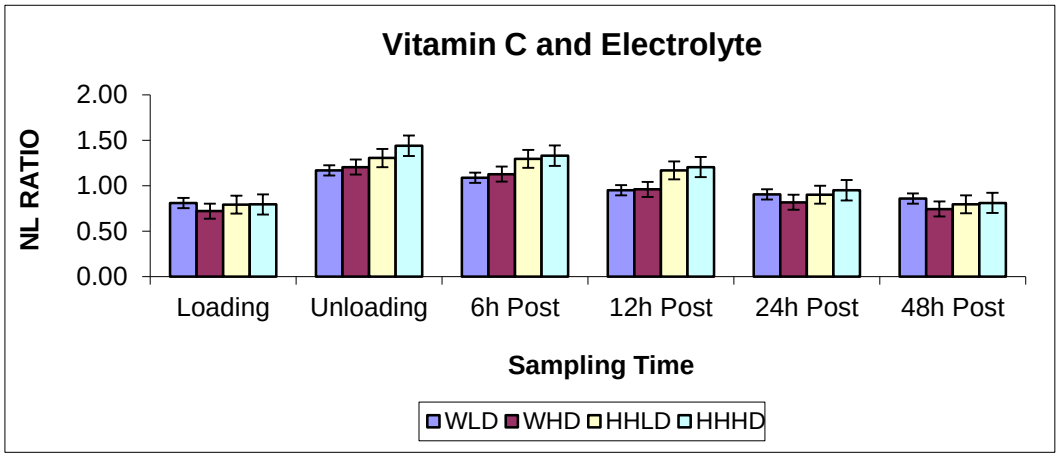

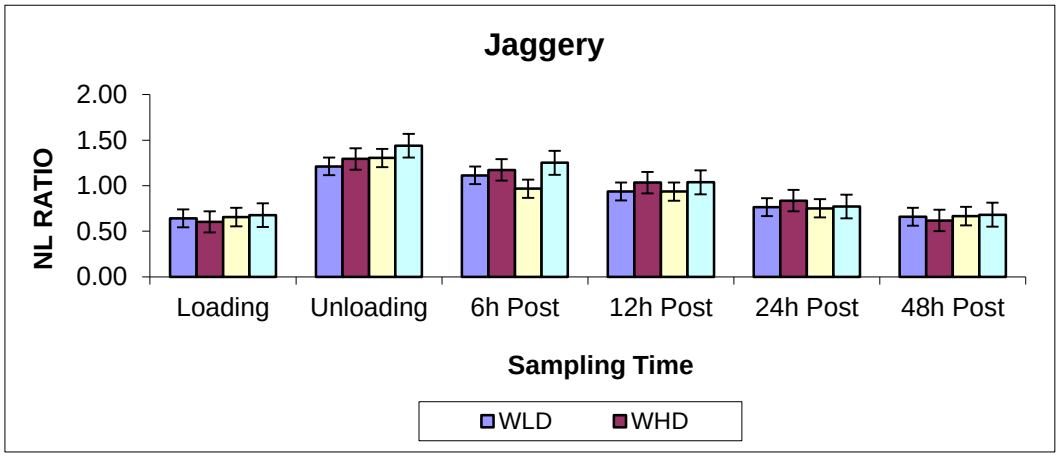

Fig. 4: Average values of neutrophil lymphocyte ratio in goats transported at different flocking densities during winter and hot humid seasons

The change in WBC counts may be as a result of the presumed sudden release of glucocorticoids during handling and loading, which is responsible for the trafficking and release of WBC from the bone marrow (Urban-Chmiel, *et al*., 2009). Increase in total leukocyte count due to transportation is also supported by

other workers (Ambore *et al*., 2009). Similarly increased total leukocyte count concentrations were obtained in pigs (Plyaschenko and Sidorov, 1987).

In all the groups, in both the seasons, higher values of neutrophils (P<0.05) were observed in goats with hfd in comparison to lfd goats just after unloading. In lfd goats, in group I, the values of neutrophils were higher in winter season as compared to goats during hot humid season whereas the values of neutrophils were higher in hot humid season than winter season in group II and III. Hfd goats of group III, exhibited maximum neutrophil value during both season. The reason might be transportation in current study can be due to 1) demargination of neutrophils 2) delayed apoptosis of neutrophils 3) stimulation of stem cells by growing factors like granulocyte colony stimulating factor (G-CSF).

In all the groups, in both the seasons, higher pre-transportation values of lymphocytes (P<0.05) were observed in both density group of goats. Pre-transportation values of lymphocytes in group I, in both the seasons, were higher (P<0.05) in hfd goats in comparison to lfd goats. During hot humid season, post-transportation, the values of lymphocytes were higher in lfd as compared to hfd goats. In both seasons and in all the three groups, minimum values of lymphocytes were recorded just after unloading in the high density group, which then increased to attain normal values (P<0.05) 24 hours post transportation. The values of lymphocytes remained significantly (P<0.05) low after 24 hours post transportation in all the groups in both seasons. In winter season, hfd goats of group II and group III, showed lower values (P<0.05) of lymphocytes as compared to lfd goats, which then increased to basal values (P<0.05) between 24 to 48 hours of post transportation. In hot humid season, lower lymphocytes (P<0.05) values were recorded in hfd goats in groups II and III. Hfd goats of group III exhibited lower lymphocytes values during winter season 24 hours post-transportation and were normalized at 48 hours post-transportation. The values of lymphocytes in group III remained lower (P<0.05) even after 24 hours post transportation in both flocking densities during hot humid season.

In group I, in both the seasons, higher values of NL ratio (P<0.05) were observed in goats with hfd in comparison to lfd goats. In winter season, maximum values of NL ratio were recorded just after unloading in the high density group, which then declined to attain normal values (P<0.05) 24 hours post transportation. A similar decreasing trend was observed for goats in hot-humid season also. Moreover in hot humid season, the values of NL ratio were much higher in both the flocking density groups in comparison to those of winter groups. Group III hfd goats exhibited higher NL ratio values during winter season. The values of NL ratio remained significantly higher after 48 hours post transportation in hfd goats during hot humid season.

The decrease in lymphocyte count due to transportation in current study can be due to 1) margination and redistribution of lymphocytes within lymphatic system (reticulo-endothelial system) 2) marked accelerated apoptosis of lymphocytes 3) higher serum concentration of catecholamine's, prolactin and Cortisol can also

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induce lymphocytopenia. These 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 differential leukocyte count was altered as evidenced by decreased in lymphocyte (lymphopenia) counts by 6.1%, respectively, and an increase of 11.3% in neutrophil count (Ayo *et al*., 2005; Minka and Ayo, 2007).

These results are in agreement with Rajion *et al*. (2001) who reported that the NL ratios were increased immediately following the road transportation and continued for 6hr and later on 12 hr rest NL ratios return to pre-stress values. Ascorbic acid a inhibitory role on cortisol, AA has also been reported to be a chain-breaking antioxidant, involved in the prevention and restriction of free radical chain formation and propagation, consequently, protecting blood cells, including neutrophils and lymphocytes from oxidative damage (Urban-Chmiel, *et al*., 2009).

Conclusion

In present study the administration of Ascorbic acid and electrolytes prior to loading and transportation ameliorated the adverse effects of loading, transportation, and high AT and RH on the immune status of the goats and overcame the stress with less response and Supplementation of vitamin C, vitamin C + electrolyte and jaggery aided in reducing transportation stress individually but vitamin C + electrolyte combination proved more beneficial in alleviating transportation stress in goats.

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