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Catecholamine and Cortisol Levels in Relation to Temperature and Transportation Stress in Goats

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Abstract

Generally, rise in stress hormonal levels can harm animal health meat quality of animals. Effect of transportation under extreme ambient temperatures was monitored in the Dhofari goats during summer (35-45 °C) and winter (15-20 °C) in two age groups, 1 and 1.5 years old. Goats were divided into control and experimental groups. Experimental group was transported for 4.5 hours in an open truck while the controlled goats remained in a shaded pen. The effect of transportation, extreme ambient temperatures and age factors caused significant stress resulted in high rise in the plasma stress hormones, adrenaline, noradrenaline and cortisol during both seasons. Adrenaline and noradrenaline plasma levels were measured using high performance liquid chromatography, while cortisol was measured using chemiluminescence immunoassay. Rectal temperature varied during the experimental procedure relative to the ambient temperature. This investigation reveals that transportation under extreme temperatures can influence the stress level hormonal levels in both which can be harmful to animal health and meat quality. In conclusion, stressful conditions must be avoided in order to prevent any side effect on goats.

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Introduction

Transported animals demonstrate a wide range of behavioural and physiological changes having the general effect of helping them survive through various stressful treatments. Even though the changes may be biologically adaptive, they are not always beneficial and are adverse in some cases. The behavioral responses are diverse and stimuli dependent. The stress response does not become harmful or pathological except when it is demanding and sustained. Only then can homeostasis be threatened and health endangered (Fuch et al., 2001).

During transportation, these animals are subjected to various physical and psychological stressors such as handling, noise, isolation, agitation, extreme temperatures (Al-Kindi et al., 2005., feed deprivation Kannan et al., 2000)., vibration, prolonged standing, humidity (Nwe et al., 1996) and high density during transportation (Knowles et al., 1998). Transport stress can also reduce meat quality. The bruising during transport leads to adverse effects on the carcass and depletion of glycogen results in a dark, dry and firm meat (Kadim et al., 2006).

These stress causing factors lead to the activation of the sympatho-adrenal-medullary axis, which results in increased catecholamines concentrations. Cortisol is the principle effector in the hypothalamic-pituitary-adrenocortical axis which affects both neurotransmission and neuroendocrine control (Fuch et al, 2001). ACTH release can be triggered in conditions such as chemical, physical, and emotional stress, such as extreme external cold or heat, toxins, overcrowding, traumatic shock, haemorrhage, infections starvation, and hypoglycaemia (Engelking, 2000) leading to increase in cortisol secretion. An increase in plasma cortisol level is considered to be a useful indicator for stress in animals (Nwe et al., 1996; Kannan et al., 2000; Kadim et al., 2006; Kannan et al, 2007; Fazio et al., 2008).

The increase in catecholamines, especially adrenaline coincides with an increase in cortisol. Studies have investigated the catecholamines levels during transportation in sheep (Parrot et al., 1994) and to a less extent in cattle (Odore et al., 2004). However, few studies on goats plasma catecholamines concentrations have been reported. One of the few studies on goats was carried by (Kadim et al. 2006), which reported an increase in adrenaline and noradrenaline concentrations during transportation.

The core body temperature is relatively constant and its daily oscillation should be within the range of 0.6-1.0 °C. Core temperature is normally indicated by the measurement of rectal temperature (Giuseppe and Giovanni, 2002). Core body temperature fluctuates diurnally (Ayo et al., 1998) but can also increase following disturbing or stressful events. In goats the rectal temperature is within a narrow range 38.5-39.7°C. Diseases or other physiological conditions like dehydration have an effect on temperature range (Cunningham and Klein, 2007).

The Aim of this study is to investigate the effect of road transportation at different time of the day, season, and age on the plasma concentrations of cortisol, adrenaline and noradrenaline. Rectal temperatures were taken at different time of the experiments to be correlated with the hormonal levels.

Materials And Methods

Experimental groups

Animals of each batch were divided randomly into two groups, 10 animals of each. The unstressed group or control, and the experimental or transportation stressed group. Each group was kept in separate lightly shaded pens, the day before the experiment commenced. The pens were spacious to allow the goats to move freely. The vehicle used for transporting the goats was not shaded except when environmental temperatures were 35-45°C in summer and 15-20 °C in winter, which could have affected the welfare of the goats.

On the day of the experiment the animals were fed early in the morning on Rhodes grass hay and commercial ruminant concentrates at 6-6:30 am. At about 7 am the first blood sample was taken from the jugular vein within one minute from each goat of the two groups. All efforts were made to avoid exciting the goats to minimize the effect of blood sampling.

After one hour, the transported group was loaded on to the truck and immediately blood was taken again to examine the acute effect of loading, and at the same time, blood was taken from the control group. After that, the goats of both groups were left to rest for another hour before the transport commenced. After an hour from taking the second blood samples, transportation started for 4.5 hours in approximately 1.5m x 2m open truck on a smooth paved road, in a built up area at a low speed 70-80 km/hr.

During the transportation period, blood samples were taken every 1.5 hours and were also taken from the control group at the same time.

The experiment was carried out with a crossover design, so two weeks later it was repeated but the places of the goats were swapped, i.e. the control group was on the truck and the transported goats were left in the farm.

Environmental temperatures in the truck and in the pen were recorded at an hour interval starting at 7:30 am till 4:30 pm (Table 1). The blood samples were collected from the jugular vein using multi sample using vacutainer needles 21g x 1" into 4.5 ml x 4 EDTA ethylenediaminetetraacetic acid vacutainer tubes for catecholamines and cortisol analyses. Plasma was separated within 2 hours by centrifugation in a refrigerated centrifuge at 4°C for 12 minutes at a speed of 1500 rpm using ALC International microcentrifuge model 4214. The plasma was then dispensed into 1.5 ml labeled microcentrifuge tubes, and stored at -80°C until assayed.

Three goats were chosen randomly from each group and rectal temperature was taken from these goats using digital thermometers. The rectal temperature was measured, immediately after taking blood by inserting a standard clinical digital thermometer MABIS Healthcare Inc, Germany, about 2 inches deep until the temperature readings stabilized.

Catecholamines

Extraction

The method used to extract catecholamines in this study was as in Al-Kindy et al. (2005) except the sample amount was changed as mentioned below. Reagents used were from Chromesystems, GmbH. Into a 2.0ml microcentrifuge tube, 75 mg of acid washed alumina Chromesystems™ Alumina 5050 Lot 4107 was placed with 750µL extraction buffer Chromesystems™ Extraction buffer 5011 Lot 4206, 800 µL of plasma and 100 µL of 12ng/ml internal standard 3, 4-dihydroxybenzyl-HBr (DHBA). Chromesystems™ Internal Standard 5004 Lot 4506. This mixture was shaken by hand for 20 minutes and then centrifuged at 5500 rpm for 3 minutes then the supernatant was aspirated. The remaining pellet was washed with 1ml washing buffer Chromesystems™ Wash Buffer 5005. The mixture was again shaken

using an autovortex for about 2 minutes and then centrifuged for 3 minutes at the same speed as before and the wash buffer was aspirated carefully. The washing process with the buffer was done three times. The catecholamines were retrieved by eluting the mixture with 240 μ L elution buffer Chromesystems™ Elution Buffer 5006 and shaken for 10-15 minutes by hand. After the shaking process the mixture was centrifuged at the same speed for 3 minutes, the supernatant containing the catecholamines and the internal standard was pipetted with care to prevent disturbing the alumina to a clean labelled microcentrifuge tube and kept in ice immediately.

Detection of catecholamines using HPLC

The extracted supernatant was analyzed the same day for catecholamines using a High Performance liquid Chromatography (HPLC) instrument Alliance Waters 2695 separation module. The HPLC instrument was calibrated using a calibration standard 5003 consisting of 5 ng/l noradrenaline, 2.5 ng/l adrenaline, 2.5 ng/l dopamine and 5 ng/l internal standard DHBA. 50 μ l of each sample was injected into the HPLC. An HPLC pump Waters® 2695 maintained a constant flow of 1 ml/min. Samples were injected into a catecholamines column Chromsystem™. Separation of the catecholamines was achieved by electrochemical detection Waters® 2465.

Reduction of catecholamines at 240 mV led to a stable baseline and clean detection of the catecholamines. The chromatograms of the calibration standard and the samples were read by Waters Empower software. The resultant peaks were converted to areas and hence to quantities of the catecholamines based on the retention time given previously by the column manufacturer and also it depended on the internal standard DHBA volume. Results were acquired and processed using Millennium software Waters. The resulted peak areas (Fig.1) indicating the catecholamines volumes were converted to ng/l using the following equation:

$$\text{Concentration of the substance in sample (pg/ml)} = \frac{\text{SUB}_{Pr}}{\text{SUB}_{St}} \times \frac{\text{IS}_{St}}{\text{IS}_{Pr}} \times \text{Factor}$$

SUB_{Pr} = Peak area of the substance of interest in the sample chromatogram

SUB_{St} = Peak area of the substance of interest in the chromatogram of the calibration standard solution.

IS_{St} = Peak area of the internal standard in the chromatogram of the calibration standard solution.

IS_{Pr} = Peak area of the internal standard in sample chromatogram.

Factors for this formula were 1200 for Noradrenaline and 600 for Adrenaline instead of 600 and 300 respectively because 100 μ l of the internal standard was mixed with the plasma instead of 50 μ l. The 100 μ l were added as in Al-Kindi 2005. procedure for catecholamines extraction. The detection limits of the HPLC were 10-10000 ng/l.

Plasma hormones

Chemiluminescence immunoassay was used for the determination of plasma concentrations of cortisol using the Beckman Coulter Access® 2 Immunoassay System and reagents (Beckman Coulter Inc, USA). This technique involves the emission of light due to the occurrence of chemical reaction and the release of energy as photons. Cortisol was measured by competitive binding immunoenzymatic assay.

Cortisol assay

The plasma required 25 μ l was added to a reaction vessel (Beckman Coulter Inc, USA) with rabbit antibody and cortisol alkaline phosphate conjugate 33600 (Beckman

Coulter Inc, USA) and paramagnetic particles coated with goat anti-rabbit capture antibody. The cortisol of the sample competes with the cortisol-alkaline phosphate conjugate for binding sites on a limited amount of specific anti-cortisol antibody. The resulting antigen-antibody complex binds to the capture antibody on the solid-phase. After incubation, the sandwich complex is separated in a magnetic field and washing using wash buffer (Beckman Coulter, Inc) removed unbound materials to the solid phase.

A chemiluminescent substrate, Lumi-Phos 530 (Beckman Coulter Inc) was added to the reaction vessel and light generated by the reaction was measured with a luminometer. The photon production is inversely proportional to the cortisol concentration in the sample. Cortisol calibrator (Beckman Coulter Inc) was used. The detection limits were 11-1655 nmol/l.

Results

Plasma Adrenaline

Treatment, season and age had an effect on adrenaline concentrations. Transport had the highest effect ($P < 0.001$), followed by age ($P = 0.002$) and season ($P = 0.003$). The mean concentration in the transported goats was higher compared to the control group (Fig. 2).

Relative to treatment, season and age interactions, in both seasons and age groups, the transported goats had higher adrenaline levels than the control groups (Fig.3). The transported goats had consistently an increase in adrenaline concentrations after the onset of the transport, except for the 1.5 yr old in cold season which the adrenaline values at the beginning of transport were below the control groups. Afterward, the adrenaline values for the transported goats began to rise after 1.5 hr. The highest adrenaline mean concentration was expressed during hot season by the transported 1 yr old goats. In hot season, both transported and control group, at the age of 1.5 yr had the lowest adrenaline concentrations compared to the same treatments in the 1 year group during cold and hot seasons (Fig. 3).

Noradrenaline

Season had a very significant effect ($P < 0.001$) on goats noradrenaline levels, unlike age ($P = 0.419$), which had no effect. Transport effect approached significance ($P = 0.054$). Noradrenaline was higher in cold season compared to hot season (Fig. 4).

In regard to treatment, season and age interactions, both control and transported goats in both age groups, had higher noradrenaline concentrations in cold season. In general, there was a significant effect ($P = 0.090$) of season on noradrenaline concentrations of both goat groups (Fig. 5).

In 1 yr old goats in both seasons and the 1.5 yr old in hot season, the noradrenaline concentrations were higher in the transported goats than the controls. In contrast, the 1.5 yr old control goats in cold season, had higher noradrenaline than the transported goats.

The response of noradrenaline concentrations during the day, varied significantly ($P = 0.022$) between cold and hot season and even between the control and transported goats in the two age groups during cold season (Fig. 5).

Cortisol

Season and age treatments had significant effect on cortisol levels ($P < 0.001$). While the cortisol concentration was higher in cold season over the hot season and in 1.5 yr goats over 1 yr goats. Cortisol concentration in the transported goats almost double compared to the control group (Fig. 6).

Relative to treatment, season and age interaction, 1yr old goats had higher cortisol concentrations during hot season while 1.5 yr old goats were during cold season (Fig. 7). In cold season, 1.5 yr old goats had almost double the cortisol concentration than in 1 yr old goats. In hot season, concentrations were similar in the two age groups and were also similar to the concentrations seen in the 1yr old goats during cold season. There was significant increase ($P=0.002$) during cold season in both groups of goats at the age 1.5 yr compared to the 1 yr. Also there was a significant ($P=0.002$) increase in hot season in both 1yr and 1.5yr (Fig. 7).

Rectal temperature

Transport had no effect on rectal temperature, but season and age had a very significant effect ($P<0.001$). The rectal temperatures were higher in hot season than cold season and in 1 yr than the 1.5 yr (Fig. 8).

In relation to treatment, season and age interaction, the mean of rectal temperatures from 1 yr old goats during hot season 39.8°C and 39.7°C for the control and transported goats respectively (Fig. 9). The lowest mean temperature was 39.0°C taken from the 1.5 yr old goats during cold season ($P=0.110$). The mean rectal temperatures were 39.1°C and 38.9°C for the control and transported respectively. The response of the two goat groups at 1 yr and 1.5 yr, in hot season was similar to the 1.5 yr in cold season, but different to 1 yr goats in cold season; as the rectal temperatures between the control 39.0°C and the transported 39.6°C were different in that age at that particular season (Fig. 9).

Discussion

Cortisol has been used as a reliable physiological end point for determining stress response (Nwe et al., 1996). Increase in plasma concentration of cortisol has been used as an indicator of stress in horses (Fazio et al., 2008), cattle (Odore et al., 2004; Gupta et al., 2007), sheep (Cockram et al., 1997) and goats (Nwe et al., 1996; Kannan et al., 2000; Aoyama et al., 2005; Kadim et al., 2006).

The normal range of plasma cortisol in goats is 57-63 nmol/l (Kaneko, 1997). Even though investigators agreed that transported animals exhibit an elevated cortisol concentrations compared to control animals, it has also revealed that this increase is variable depending on certain factors, such as genotype, sex, physiological state, population density and environmental temperature. Similar studies were reported by Nwe (1996) on Japanese goats and Kadim et al. (2006) on three breeds of Omani goats.

This study revealed that all the transported goats were stressed, as plasma cortisol concentrations were doubled during transportation. Nwe et al. (1996) reported that the cortisol concentrations increased significantly, three times higher than the baseline, within 1h. The stress susceptibility of an animal also varies with age and breed (Kent and Ewbank, 1986). This is in agreement with the present study in that cortisol concentrations were found to be significantly higher during cold season and in older goats. With regards to season, there is a possibility that Omani goat adapted to hot weather, so when they are transported during cold season they are actually subjected to both transportation and cold stresses. The age effect was more obvious during cold season, as the mean cortisol concentration was higher for 1.5 yr transported goats compared to 1yr old.

Cortisol levels in most species tend to be higher in the morning than in the afternoon (Broom, 2003). In the present study this phenomenon was observed in control goats. In the transported goats, generally the cortisol increases to maximum levels at 1.5h, then decreases

after 4.5hr of transport. The decrease in the cortisol values in the transported goats towards the end of the transported period could be either a natural tendency to fall similarly to the control group or could be the result of negative feedback of cortisol on ACTH resulted of the effect for long period of transport (Squire, 2003).

Catecholamines and cortisol are essential components of adaptation to stress. Catecholamines cannot facilitate the stress response alone without cortisol (Murray et al., 1996). It has been reported that many stressors can induce the release of the catecholamines, which may reduce immunity in the body (Odore et al., 2004).

The changes in plasma adrenaline in this study indicate that transportation caused a significant increase in adrenaline levels. The adrenaline levels measured after the onset of transportation was three times higher in 1 yr old goats during hot season and double the concentrations in 1.5 yr old transported in cold season compare before the start of transportation. The mean adrenaline concentration for 1 yr old transported goats, during hot season was significantly higher than the control groups. This also indicates that transported goats had almost three times higher adrenaline concentrations than the control goats during hot season. In the present study, the elevation of adrenaline in transported Dhofari goats is in agreement with the results reported by (Kadim et al. 2006). In addition Parrot et al. (1994) reported an increase in adrenaline in adult sheep subjected to physical stressors such as transport simulation, standing in water or handling which is similar to our data before the transportation process.

During cold season, the 1.5 yr old control goats had adrenaline levels similar to the transported goats. This implies that the effect of cold stress was strong enough to cause an increase in adrenaline levels in control goats close to the transported goats.

The 1.5 yr old goats, transported in cold season, had double adrenaline concentrations compared to transported 1.5 yr old goats during hot season. In addition, during cold and hot seasons, the transported 1 yr old goats had higher adrenaline concentrations compared to the transported 1.5 yr old goats in both seasons. This may indicate that higher adrenaline concentrations expressed in younger goats. This could be that younger goats were more sensitive to transportation stress than older goats which triggered the release of adrenalin. Mean adrenaline concentrations in both seasons were higher in 1yr old compared to 1.5 yr old. This may be due mainly to the influence of temperature and sensitivity of the goats to stress.

The noradrenaline concentrations during transportation were not significant. The noradrenaline concentrations in 1 yr old transported goats were double to that of control group during hot season, while in cold season they were about one third higher. In addition there was no significant effect of age, which suggests the significant effect of cold season, increasing noradrenaline concentrations.

Previous studies (Odore et al., 2004) reported that catecholamines generally, and particularly adrenaline, are an indicator of stress. Thus high concentrations obtained in the current study may be a reflection of stress during transportation. The data from these studies also reported that noradrenaline increases mainly during physical activity. The increase observed in the present study could be the result of continuous standing for about 4.5 h throughout transportation.

Moreover, there was variability between the groups and between time points but overall there were consistent effects. Based on this, adrenaline can be a good indicator of stress when transporting goats for long time.

In this study, rectal temperatures in goats were between 38.5-39.7°C. A substantial response of the adrenal cortex may increase body temperature which is usually 1°C. Temperature range

38.9-40°C is normal in goats (Broom, 2003). Extensive research has been done on the effect of transportation on rectal temperature in sheep and cattle but limited in goats living in extreme ambient temperatures above 30°C, like the conditions in Oman.

Transporting Dhofari goats for 4.5 h at ambient temperatures 15-26°C. during cold season and 27-42°C. in hot season did not have a significant effect on the rectal temperature. The overall range of rectal temperatures in transported goats were 38.6-40°C. and 38.9-40.1°C. in control goats. This could be related to low crowding and well ventilated vehicle during transportation. Based on this data, the goats rectal temperatures remained stable in spite of the extreme ambient temperature changes. These results are in agreement with Rajion et al. (2001) who reported no variation in body temperature before and after transportation. Rectal temperature in goats is not a reliable indicator of stress during transportation. Hot season could be stressful specifically to 1 yr goat transported goats.

In conclusion, monitoring hormonal levels in goats during transportation in hot and cold temperatures revealed that stress hormonal levels rose significantly over the controls which may indicate that transportation and temperature are important factors in inducing stress in goats.

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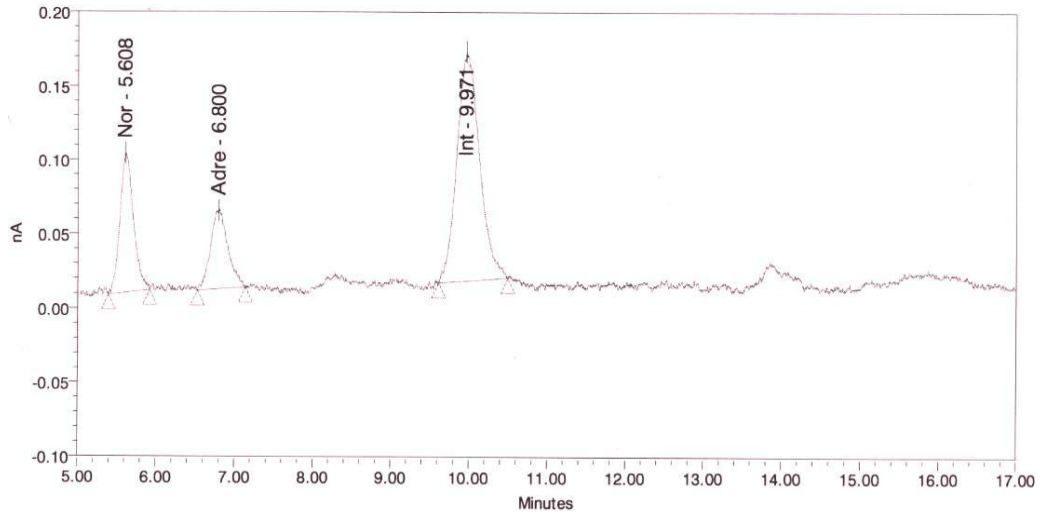


Fig.1. The peaks produced by the HPLC software, correlate to the catecholamines volumes.

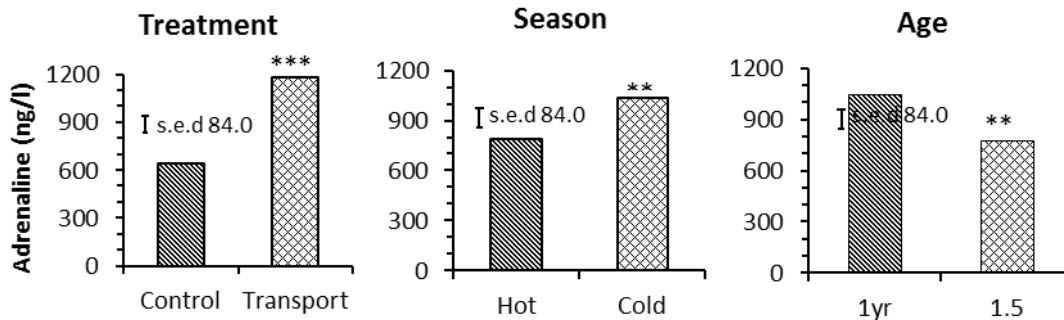


Fig.2. Grand mean of plasma adrenaline concentrations from all control and transported male

Dhofari goats, during hot and cold season and at the age of 1 year and 1.5 year. ** P<0.01, *** P<0.001 n=62 per treatment group.

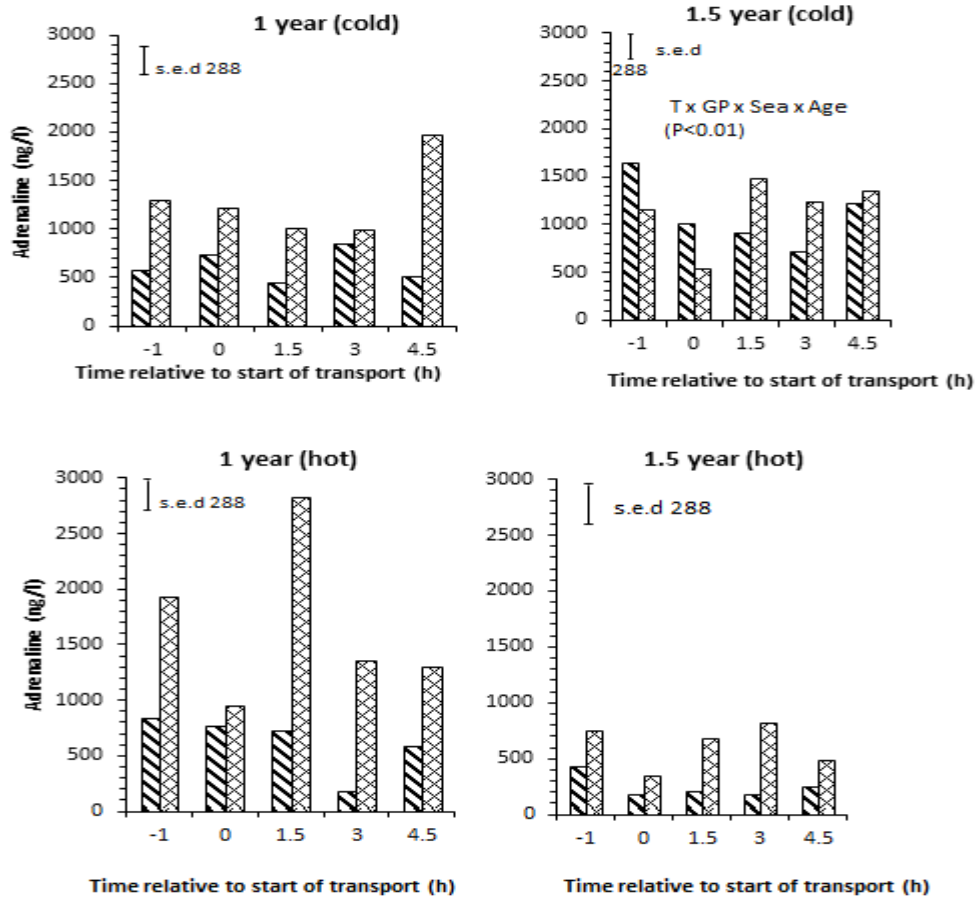


Fig.3. Plasma adrenaline concentrations in transported and control male Dhofari goats. During cold season in 1 year and 1.5 year and during hot season in 1 year and 1.5 year old goats. 1h before road transport -1., immediately after loading 0. and 1.5, 3, and 4.5hr post onset of transport in an open truck. T: time, GP: treatment group, Sea: season. n=62 per treatment group.

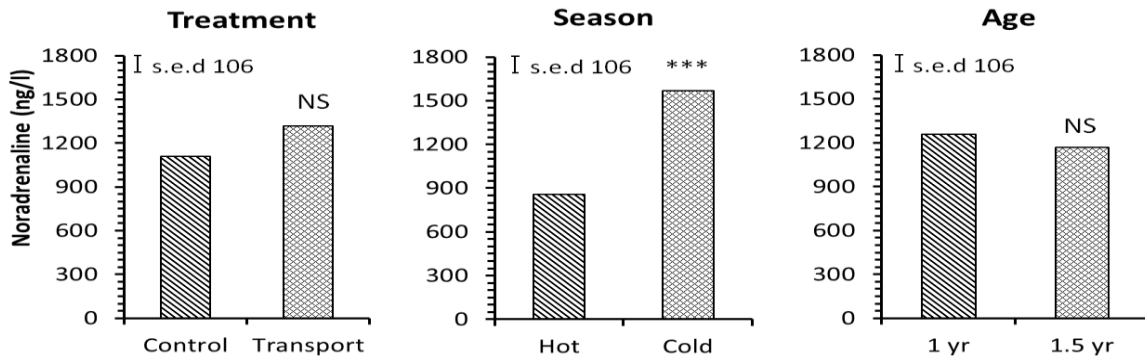


Fig.4. Grand mean of plasma noradrenaline concentrations from all control and transported male Dhofari goats, during hot and cold season and at the age of 1 year and 1.5 year. NS: not significant, *** $P < 0.001$ $n = 62$ per treatment group.

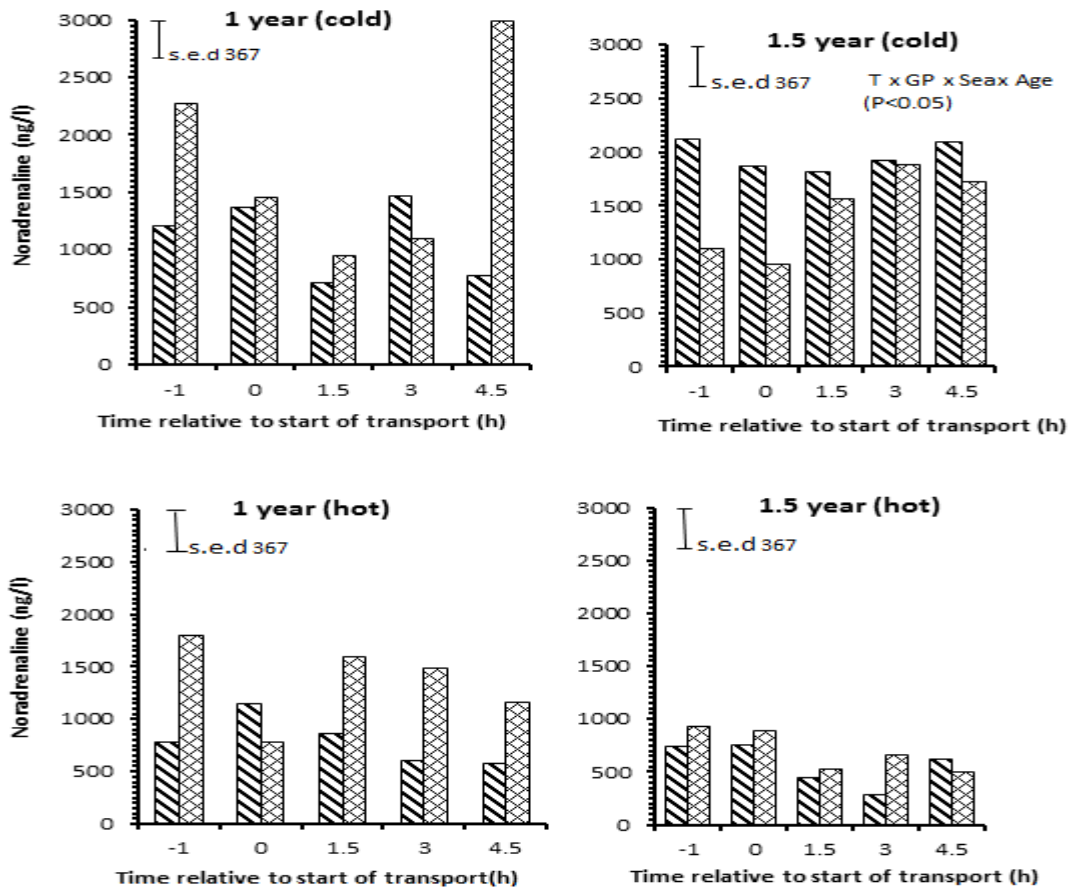


Fig.5. Plasma noradrenaline concentrations in transported control and Dhofari goats. During cold season in 1 year and 1.5 year, and during hot season in 1 year and 1.5 year old goats. 1h before road transport -1., immediately after loading 0. and 1.5, 3, and 4.5hr post onset of transport in an open truck. T: time, GP: treatment group, Sea: season. $n = 62$ per treatment group.

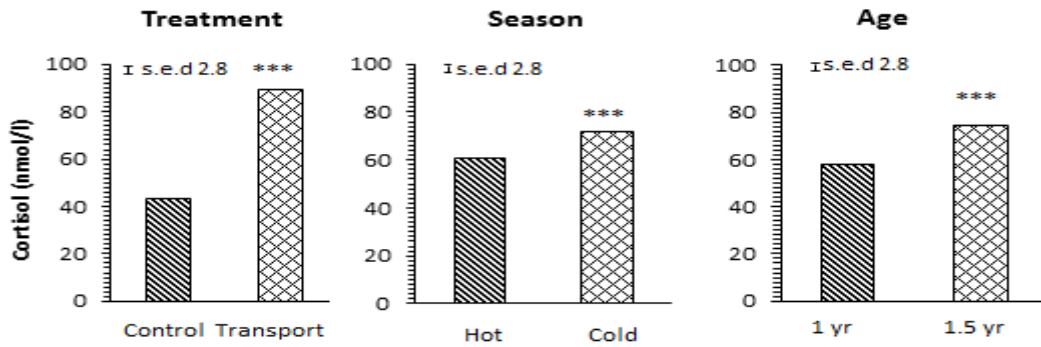


Fig.6. Grand mean of plasma cortisol concentrations from all control and transported and control male Dhofari goats, during hot and cold season and at the age of 1 year and 1.5 year. *** P<0.001 n=62 per treatment group.

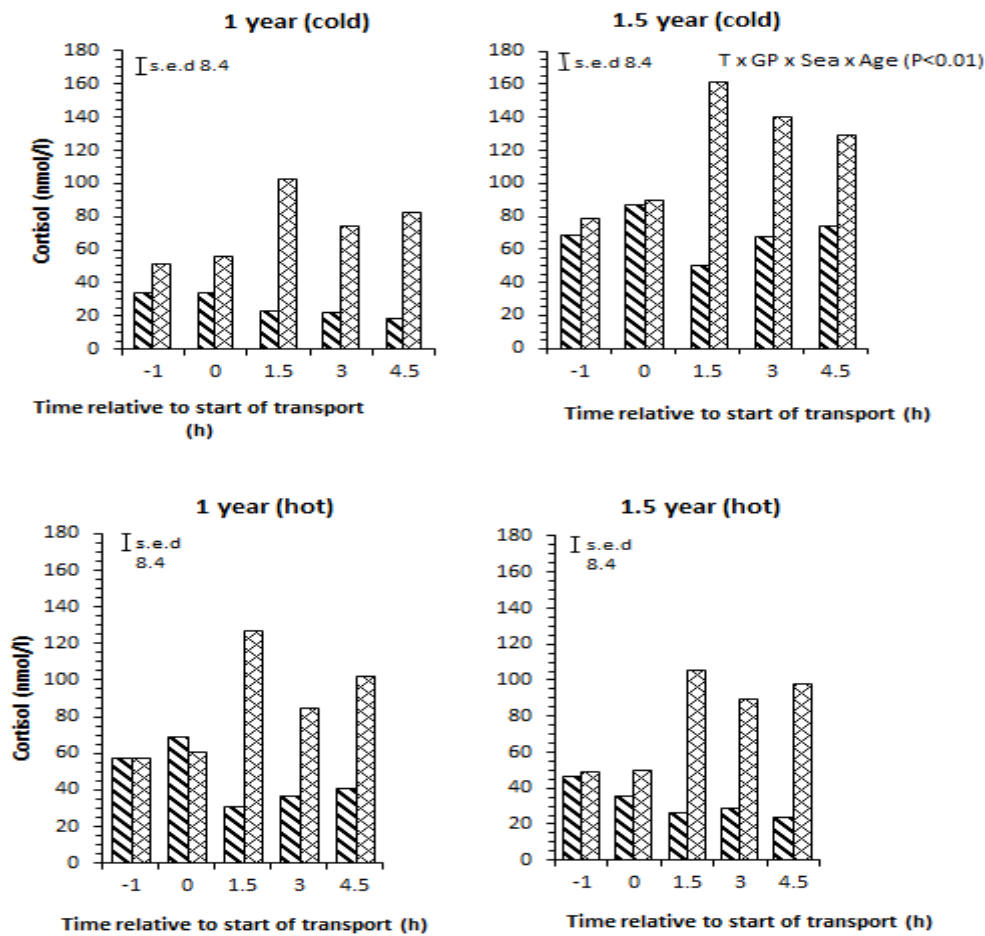


Fig.7. Plasma cortisol concentrations in control and transported male Dhofari goats. During cold season in 1 year and 1.5 year, and during hot season in 1 year and 1.5 year old goats. 1h before road transport -1., immediately after loading 0. and 1.5, 3, and 4.5hr post onset of transport in an open truck. T: time, GP: treatment group, Sea: season, n=62 per treatment group.

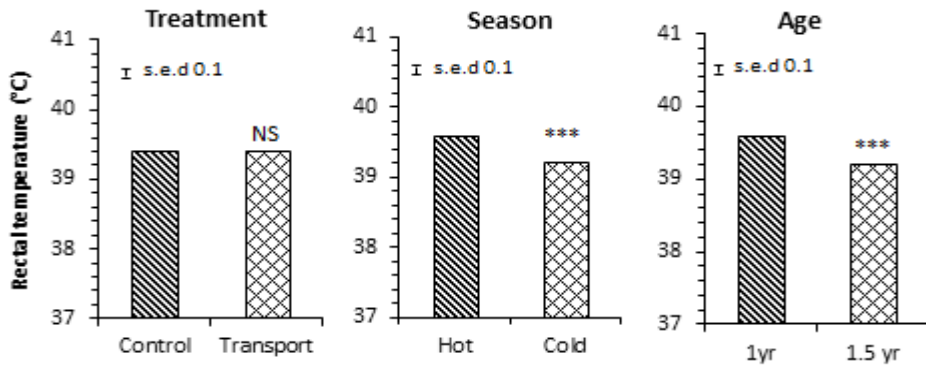


Fig.8. Grand mean of the rectal temperature taken from all control and transported male Dhofari goats, during hot and cold season and at the age of 1 year and 1.5 year. NS: not significant, *** $P < 0.001$ $n = 24$ per treatment group.

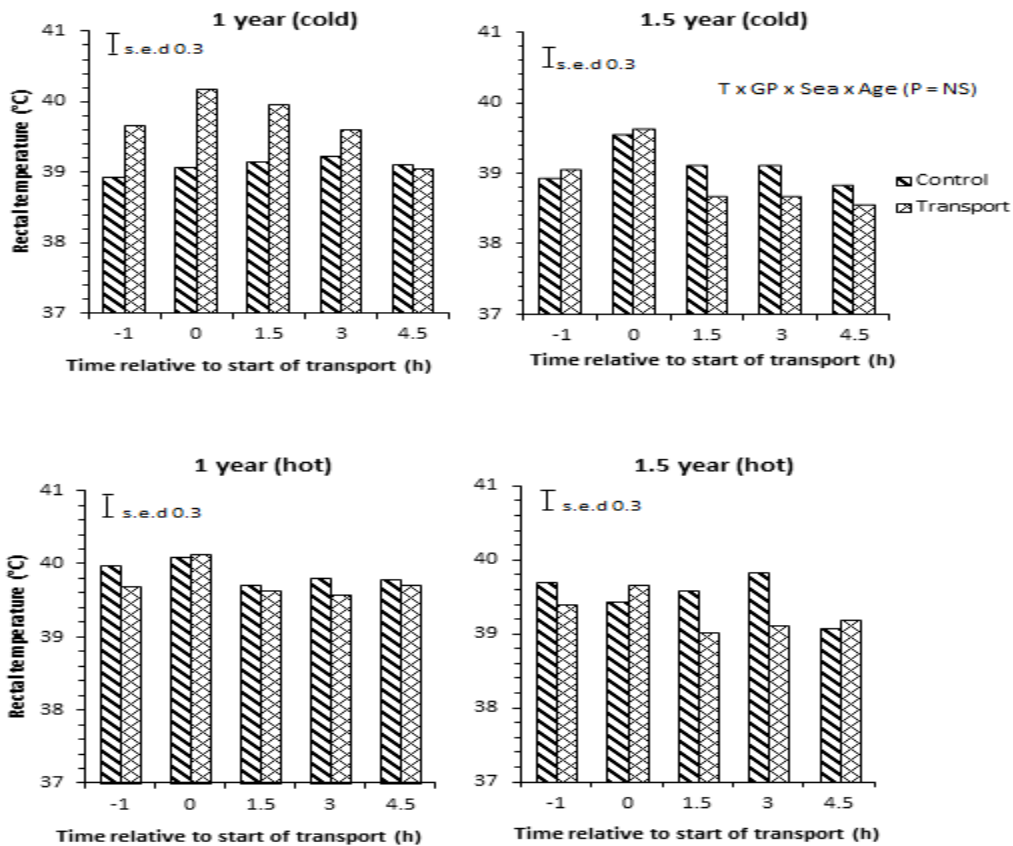


Fig.9. Rectal temperature in transported and control male Dhofari goats. During cold season in 1 year and 1.5 year, and during hot season in 1 year and 1.5 year old goats. 1h before road transport -1., immediately after loading 0. and 1.5, 3, and 4.5 hr post onset of transport in an open truck. T: time, GP: treatment group, Sea: season, NS: not significant $n = 24$ per treatment group.

Table 1:Environmental temperature at an hour interval, during the blood sampling days measured in °C. * No data collected.

Day		7:30 am	8:30 am	9:30 am	10:30 a m	11:30 am	12:30 pm	1:30 pm	2:30 pm	3:30 pm	4:30 pm
1	On truck	*	*	*	*	*	*	*	*	*	*
	In farm										
2	On truck	23	22	25	*	*	27	*	27	*	26
	In farm	23	22	23.5	24.5	25	26	26	26	25	25
3	On truck	36	38	40	41	41	39	40.5	40	40	40.5
	In farm	36	38	40	43	44	41	41	42	40	40
4	On truck	36	39	40	41	40	42	39	41	39	39
	In farm	36	39	40	41	42	41	40	40	39.8	39.8
5	On truck	37.5	40	41	38	38	32	38.5	39	37.5	*
	In farm	37	39	41	37.5	38	38	37.9	36	36	*
6	On truck	36	39	40	38.5	38	36	39.5	38	39.5	*
	In farm	36	37.5	39.5	40	36.5	37	38.9	39	37.5	*
8	On truck	19.5	22	25	26.5	27	31	28.5	28.5	26	25.8
	In farm	19.5	22	25	25	27	28	27	27	26	25
9	On truck	21	25	27	28	27.5	26	27	26.5	27	26
	In farm	21	25	27	27	28	26	27	26	26	26