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Physiological Response of Two Age Groups of Omani Sheep to Short Road Transportation in Relation to Circulating Levels of Gonasotropins, Cortisol, Thyroid Hormones, Sex Steroids and Plasma Chemistry

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Abstract: Two age groups of Omani sheep were subjected to a short period transportation under two sets of stress conditions. Blood samples were collected by jugular veinpuncture just before slaughtering and the degree of stress was related to hormone levels and blood chemistry. Chemiluminescence's immunoassay technique was used to determine levels of cortisol,, gondadotropin (FSH, LH), Testosterone (T), Thyroid Stimulating Hormone (TSH) and Thyroid Hormones (T3, T4). Adrenaline, noradrenaline, and dopamine levels were determined by HPLC with electrochemical detection. In addition, plasma values of cholesterol, total protein, blood urea nitrogen (BUN3), lactate, CO_2 Mg^{++}, PO_4^{++}, Ca^{++} and uric acid were also analyzed. A total of 24 sheep were used, 12 at age of 6 months and 12 at 12 months. Each age group was randomly divided equally into control and experimental. The control sheep from each age group were transported using open truck (3X2 m) three days before they were slaughtered. The sheep were loaded at 7:00am on a 2 h journey with heavy traffic under air temperature, which varied between 30-31°C. The end of the journey, each age group was kept weparat3ly in a lairage of a commerical slaughterhouse, with food and water ad libitum. The experimental group was subjected to the same transportation condition and with approximately the same temperate range except they were transported the same day they were slaughtered. The sheep in both age groups, which were transported the same day and then slaughtered at the end of the journey (experimental) had significantly higher cortisol value ($p<0.001$) than the control sheep of both ages. There was no significant difference in the catecholamine values between the experimental and control of both age groups but adrenaline and dopamine levels in the 12month sheep were significantly higher in both categories ($p<0.001$) than the 6month sheep. There was no clear trend in the hormone profiles and the values generally exhibited mixed results. For example FSH, TSH and T3 values wer3e significantly ($p<0.05$) higher in the 6-month control over the experimental, but in the 12month sheep the results were the opposite. In the blood parameters, there was also unclear trend in values. In the 6 month control, at least all the parameters were significantly lower ($p<0.05$) than in the experimental but the results were opposite in the 12 month sheep. Th3ere is some indication that the Omani sheep which transported on the same day demonstrated a degree of physiological stress which is based on higher values of cortisol. This probably is not caused by transportation alone but also on loading and unloading as well.

Key words: Omani sheep, circulating levels, gonasotropins, sex steroids, plasma chemistry

INTRODUCTION

Sheep account for approximately 15% of the livestock population of Oman. They are a valuable source of protein for majority of Omani people. Omani sheep are adapted to extreme heat and limited vegetation in an arid environment. As a meat animal, their body fat content (15%) is half of that of Australian sheep (Mahgoub and Lodge, 1994).

Several measures can be used to evaluate the welfare state of the animals. These include behiavour, biological functions related to stress physiology and pathological data. These evaluations should be based on multiple indices, since no single measure can unequivocally determine the stress level. The measurement of endocrine parameters is probably the most widely used especially cortisol and catecholamines, which have been equated with stress levels (Parrott et al, 1994).

Live animals in the Sultanate of Oman are routinely transported between farms and markets for slaughtering. They are usually transported in open trucks over a distance of about 100 km. During transportation, animals may be subjected to a variety of physical and psychological stimuli. These include crowding, noise,

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handling, isolation, agitation and extreme temperatures. There is substantial research work on the effects of handling and transportation of cattle, pigs and poultry (Rollin, 1995), but little work has been carried out to assess the effects of stress in transported sheep and goats especially under hot and semiarid conditions.

Transportation of animals is generally reported as a stressful event (Schrama, et al., 1994; Fraser and Broom, 1997). Sheep of temperate origin show seasonal adaptation that promotes survival and reproductive characteristics, probably related to immune system controlled by photoperiodic information and therefore, there may be differences in response to stress between temperate and tropical sheep (Nelson and Drazen, 2000). Stress in animal husbandry is related to changes in hormonal levels and blood chemistry as well as behavioral reactions. Various stressors can activate the pituitaryadrenal axis (Dantzer and Mormede, 1983).

In farm animals exposure to different environmental stressors elicit various physiological and psychological changes. Some of these are emergency reactions which are related to the activation of the adrenomedullary system and resulted in release of catecholamines which mobilize for short response to achieve metabolic adjustments (Dantzer and Mormede, 1983). The other is the general adaptive syndrome describe by Selye (1936) involves the activation of the pituitary-adrenal cortex axis resulted in release of corticosteroids which in turn extend the metabolic effects of catecholamines and Adredrocorticotropic Hormone (ACTH).

The purpose of this investigation ins to gain some knowledge about the effect of some stressful factors induced by the farmers on the sheep prior to slaughtering. Some of are handling, rounding, loading, unloading and transport. In addition, the seep occasionally are exposed to other factors such as extreme heat and fear such as keeping the animals in lairage near slaughter houses. The experiment in this study are abased on the actual routine essential daily preparations and practices that are necessary to get farm animals ready for slaughtering.

In this investigation the focused on two frequent stressors that the sheep encounter. These are transportation and keeping gin lairage. Associate with these two are other stressors mentioned above.

This study was carried out to investigate the impact of short road transport on cortisol, catecholamines, sex steroids, thyroid hormones, gonadotropins and blood chemistry in Omani male sheep. These physiological parameters have been proposed as sensitive indices of physiological stress response in animals that encountered short-term welfare problems such as handling and transportation (Broom and Johnson, 1993).

MATERIALS AND METHODS

Animals: Twenty-four native Omani male sheep ranging in body weight between 30-33 kg were used in this study. These included twelve sheep of 6 months of age and 12 sheep of 12 months. One month prior to the experiment, the animals had been dipped in a solution of Gematox to eliminate ectoparasites. All animals were injected every two months prior to the experiment with 0.5 mL^{-1} Ivomec as described by the manufacturer 0.5 mL^{-1} per 25 kg of body weight for control of internal and external parasites. A commercial concentrate (Al–Dhariat Animal Feed Company, Barka, Sultanate of Oman) was offered to the animals in their pens at 150g per head daily. Fresh water and Rhodes grass (*Chloris gayana*) hay were also provided as *ad libitum*.

Treatment: The sheep from each age group were divided randomly into control and 13 experimental. Three days prior to slaughter, the control sheep from each age group were transported in the morning (07:00) on smooth roads in an open truck (3X2 m) at ambient temperatures of 30 –31°C for two hours with several stop signs. They were then kept in a pen under shade in a lairage (10X10 m) at the Central Slaughterhouse at Baushar. Feed and water were available throughout the 3-day waiting period. The experimental sheep were subjected to the same transportation conditions except they were transported the same day they were slaughter. Prior to blood sampling and slaughter (10:30-11:00 am), the temperature was 37.5°C.

Blood Sampling and processing: Blood samples were collected from the jugular vein using 7 mL^{-1} vacutainer (Beckton Dickson) tubes containing sodium heparin for all the treatment parameters except catecholamines where EDTA tubes were used. Blood was collected within one minute from each animal with minimum disturbance to avoid excessive stress. Blood samples were kept in ice, and plasma was separated within 2 hours of collection by centrifugation at 5°C for 10 min. at 3000 rpm. The plasma was then dispensed into 1.5mL^{-1} Eppendorf tubes and stored at –80 °C. Chemiluminescence immunoassay was used for the determination of plasma hormonal levels using a Beckman Coulter Access 2 immunoassay system and reagents. (Beckman Coulter, Inc.). For the extraction of plasma catecholamines (all reagents Chromsystems GmbH), 75mg of acid washed alumina was placed into a 2.0 mL^{-1} Eppendorf tube and then 750 iL of extraction buffer, 750 μL of plasma, and 100 μL of dihydroxybenzoic acid (DHBA) standard 12 $ngmL^{-1}$ were added. This mixture was shaken for 20 minutes using an autovortex and then

Table 1: Methodology of blood plasma analysis parameters using Beckman Synchron CX Systems.

Methodology	Parameter	Reaction Mechanism	Detection
Colorimetric	Calcium	Ca-Arsenazo III Complex	Abs(650)
Potentiometric	Sodium	Ion selective electrode	Potential
Potentiometric	Potassium	Ion selective electrode	Potential
Potentiometric	Chloride	Ion selective electrode	Potential
Enzymatic	Cholesterol	Cholesterol esterase/Cholesterol oxidase withproductionofperoxide peroxidas eadditionto produce quinoneimine	Abs(520)
EnzymaticUrea	Urea	seaddition to form ammonium ion	ΔMho
Enzymatic	Uric acid	Uiricase to produce allontoin and hydrogen peroxide Hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP)and 3.5-dichloro-2-hydroxybenzene sulfate (DCHBS) in presence of peroxidase to form a quinonimineAbs	(520)

centrifuged at 5500 rpm (ALC International microcentrifuge model # 4214) for 3 minutes and then the supernatant aspirated. The resulting pellet was washed with 1 mL^{-1} washing buffer. The mixture was then shaken as before, centrifuged for 3 minutes, and the wash buffer carefully aspirated. The washing process was repeated three times. To retrieve the catecholamines from the alumina, the pellet was eluted using 240 μL elution buffer and shaken for 7 minutes, using the autovortex, centrifuged at 11500 rpm for 5 min. and the supernatant containing the catecholamines and internal standard was pipetted carefully to a clean vial without disturbing the alumina layer. This supernatant was immediately analyzed using a HPLC with electrochemical detector (Waters 600S, 464 ECD and 717 Autosampler). Results were acquired and processed using Millenium^{32} software (Waters). Additional methods for specific analytes are listed in Table 1.

Statistical analysis: The differences in the levels of the various hormones released by the control and the experimental sheep were contrasted using a two way analysis of variance (ANOVA). Other comparisons between results obtained within the two age groups of sheep were made using a two sample t-test. All probability values were one-tailed, and significant differences were determined at $p<0.05$. All computations were carried using the statistical software SPSS (1997).

RESULTS

Hormones: The 6 month control sheep demonstrated significantly higher FSH and LH values over the 12 months controls ($p<0.05$) while TSH,T, T_3 and T_4% l3f3ls were higher in the 12 month controls ($p<0.05$) (Table 2). In the 6 month sheep (controls vs. treated), the following hormones were significantly higher ($p<0.05$) in the control group over the experimental: FSH, LH, TSH and T_3 but T_4 was significantly higher ($p<0.05$) in the experimental sheep. There was no change in the T values (Table 2). In the 12month sheep (control vs. treated0: FSH, TsH and T_3 levels were significantly higher ($p<0.05$) in experimental than controls. However, T levels were significantly lower in the experimental ($p<0.001$) while LH and T_4 values remained unchanged.

Blood parameters: Blood parameters vales were compared in the control sheep of the two age groups. There was no change in cholesterol, BUN3, lactate, $CO_2$and uric acid values, however PO_4 values wer3e significantly higher in the 6 mot control ($p<0.001$), the following values were significantly higher in the12 months control sheep than in the 6 month controls: Mg^{++}, Ca^{++} and total protein ($p<0.001$) (Table 3). In the 6 month sheep (controls vs. experimental), except for the uric acid valu4es, which remained unchanged, all the other plasma parameters were lower in the experimental group than in the control ($p<0.001$) (Table 3). In the 12month sheep (control vs. experimental), the results were o pposite to that of the 6month (control vs. experimental sheep except for cholesterol and uric acid values with no significant changes (Table 3).

DISCUSSION

The Omani sheep transported the same day of slaughtering (experimental) were under stress based on higher values of cortisol over the sheep that were transported 2 h (control). Apparently the lower hormone values in the control sheep may signify that there was sufficient time for recovery from stress if we assume that the controls sheep attained the same cortisol levels when they first unloaded from the truck after the journey. In other study, sheep that were subjected to loading, penning and transport, demonstrated large increase in plasma concentrations of cortisol during the first 180 min but afterwards the hormone relases was minimal (Brook et al. 1996). In this study, the transport journey lasted for 2h and it is possible that there was enough time for the controls to recover from the transport stressor 72 h later, as indicated by the low values of the cortisol. Moreover,

Table: 2 Mean (SE) of hormone levels in 6 and 12 month Omani sheep subjected to transportation 72 h before slaughter (control) and transported on the same day of slaughter (experimental)

	6-Month		12-Month	
Hormone	Control	Experimental	Control	Experimental
FSH (IU/L)	0.09±0.091	0.00±0.000	0.00±0.000	0.43±0.114
LH (IU/L)	0.68±0.128	0.08±0.142	0.45±0.174	0.37±0.161
TSH (nmol/L)	0.10±0.041	0.03±0.046	0.16±0.056	0.21±0.052
Estradiol (nmol/L)	0.07±0.007	0.03±0.008	0.04±0.009	0.06±0.008
Progesterone (nmol/L)	0.57±0.187	0.50±0.202	0.33±0.202	0.86±0.187
Testosterone (nmol/L)	3.00±3.740	3.25±3.740	14.27±3.053	6.06±2.830
T3 (nmol/L)	5.05±0.360	4.41±0.400	6.63±0.487	9.80±0.451
T4 (nmol/L)	12.96±0.639	15.58 ±0.707	13.65±0.866	13.21±0.802

Table 3: Mean (± standard error) of plasma components in 6 and 12 month Omani sheep subjected to transportation 72 h before slaughter (control) and transported on the same day of slaughter (experimental)

	6-Month		12-Month	
Hormone	Control	Experimental	Control	Experimental
Cholesterol (IU/L)	1.54±0.095	1.40±0.120	1.57±0.109	1.49±0.101
BUN3 (IU/L)	0.13±0.125	0.00±0.000	0.10±0.100	0.21±0.114
Lactate (nmol/L)	0.13±0.125	0.00±0.000	0.10±0.100	0.21±0.114
CO_2 (nmol/L)	0.13±0.125	0.00± 0.000	0.10±0.100	0.21±0.114
Mg (nmol/L)	0.90±0.028	0.84±0.035	0.96±0.032	1.04±0.029
PO_4 (nmol/L)	3.17±0.133	2.53±0.169	2.14±0.154	2.40±0.143
Ca (nmol/L)	2.36±0.053	2.29±0.067	2.68±0.061	2.82±0.057
Uric acid (nmol/L)	0.02±0.001	0.02± 0.001	0.02±0.001	0.02±0.001
Total protein (nmol/L)	62.63±1.109	57.12±1.403	80.00±1.281	84.57±1.185

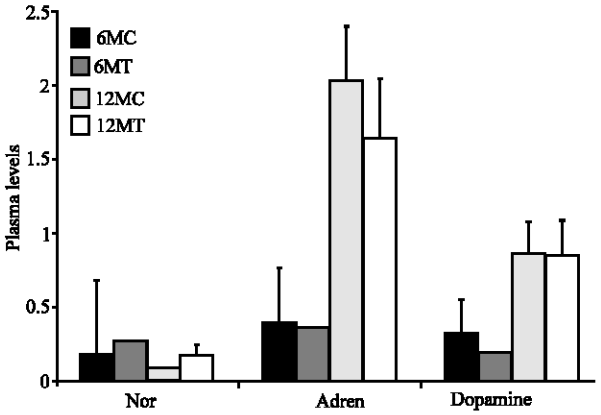

Fig. 1. Plasma catecholamine levels in Omani sheep (mean ± standard error) under two different treatments (experimental vs. control)
Control: sheep of both age groups were transported 72 h prior to slaughter
Experimental: sheep of both age groups were transported on the same day prior to slaughter 6MC= 6 month control; 6MT= 6 month experimental; 12MC=12 month control; 12MT; 12 month experimental

comparing the cortisol concentrations in both studies, it revealed that Omani sheep had significantly higher cortisol levels in both the experimental and the control groups. Only during the first 180 min of their experiment

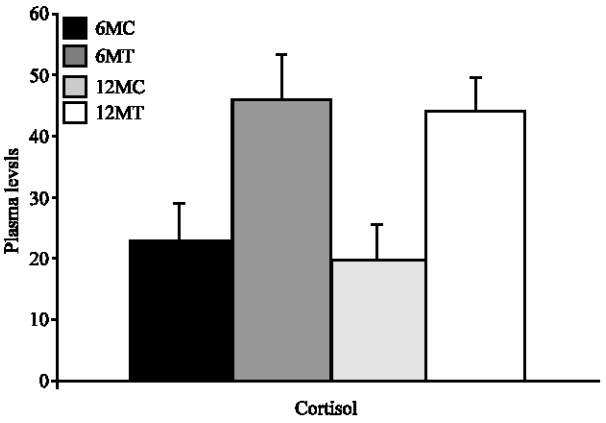

Fig. 2. Plasma cortisol levels in Omani sheep (Mean±SE) under two different treatments (experimental vs. control)
Control: sheep of both age groups were transported 72 h prior to slaughter
Experimental: sheep of both age groups were transported on the same day prior to slaughter 6MC= 6 month control; 6MT= 6 month experimental; 12MC=12 month control; 12MT; 12 month experimental

(Broom et al, 1996), the cortisol levels were comparable to the levels of Omani sheep.

The Omani sheep are under high temperature and humidity during most of the year and therefore, they may frequently maintain high relies of adrenocorticoid hormone for combating stress.

Adrenaline and dopamine w ere significantly higher in the 12 month experimental and control sheep over the 6 months sheep. However, within each age group, the adrenaline, dopamine and noradrenaline concentrations were not significant (Fig. 1) . Parrott et al. (1994) reported that adrenaline was released 10 min after the seep were subjected to transport simulation and isolation. Subsequently there was a significant rise in the hormone levels. In the Omani sheep, there is a possibility that the rise in catecholamine values were brief and in pulsatile mode and we might have missed the rise when the blood samples were taken.

In other circulating hormones, the results indicated that there were some trends of consistency in some data and inconsistency in others when the hormone concentrations between the control of the two age groups or between the experimental and control within each age group were compared. In the age group, the 12-month control had significantly higher TSH, thyroid hormones and T over the 6month control. However, the gondadotropin results were the opposite.

In the 6 and 12month sheep (control vs. experimental), the results within each age group showed that FSH, TSH and T_3 were significantly higher in the experimental over the control. However, the rest of the hormones were either showed reverse results or remained unchanged.

CONCLUSIONS

Although the data from this study did not reveal any significant changes in hormonal and blood parameter levels between experimental and control groups, these results are of value for future studies. Extensive experimentation on stress in Omani sheep are needed not only in understanding the degree of stress under different hormonal levels but also to avoid harmful stress condition that can affect meat quality. One major environmental stressor that needs to be thoroughly investigated is the heat stress, since air temperatures in a man exceed 30°C during most of the year. Further investigation on Omani sheep must involve hormonal levels, plasma parameters, heart and respiratory rates; body temperature must be monitored under different sets of heat exposures. This line of research will be expanded in future since such research has an economical implication related to quality of Omani sheep.

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