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

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Abstract: The effect of short road transportation in two age groups of Omani male goats was evaluated to assess their physiological response to stress in relation to circulating levels of catecholamines (adrenaline, noradrenaline and dopamine), Thyroid Stimulating Hormones (TSH), thyroid hormones (T_3, T_4), gondadotropins (FSH, LH) and testosterone (T). In addition, plasma parameters cholesterol, total protein, Blood urea nitrogen (BUN), uric acid, lactate, Mg^{++} PO_4^{--} Ca^{++} and CO_2 were also analyzed. Thirty seven male goats were used, 10 at 6 months of age and 27 at 12 months of age. Each age group was divided randomly into control and experimental groups. The control goats from both age groups were transported in an open truck (3 × 2 m), three days before they were slaughtered. The journey started at 7:00 am and lasted for 2 with light to heavy traffic at air temperature 30-31°C. At the end of the trip, the control goats were kept in a lairage of a commercial slaughterhouse with feed and water provided *ad libitum*. The experimental goats were subjected to the same transportation conditions except they were transported the same day they were slaughtered. Blood samples were collected from both age groups by jugular veinpuncture just before slaughtering. There were no significant differences in catecholamines, cortisol, other hormones and electrolyte values between the 6 and 12-month control groups. When the experimental data from both age groups were combined and compared with the controls of both age groups, the values remained the same expect for CO_2, BUN and lactate, which were significant. Subjecting goats to the two h road transportation as well as to other stressors such as keeping the control animals in the lairage for 72 h, did not generate a major physiological response as that indicated by the lack of significant changes in the values of the stress hormones (cortisol and catecholamines). The results of this study will be of a value form more elaborate future study on the stress physiology of the Omani goats.

Key words: Gonadotropin, sex steroid, cortisol, plasma chemistry

INTRODUCTION

Livestock production in Oman, which is mostly practiced by small holders, involves routine transportation of live animals between farms and markets for slaughtering. The distance of transportation does not exceed 100 km and is usually carried out in most of the Sultanate of Oman in open trucks. Transported animals may be exposed to a variety of physical and psychological stimuli including crowding, noise, handling, isolation, agitation and extreme temperatures. Transportation of animals is generally recognized as a stressful event[1,2]. While there is substantial study on the effects of handling and transportation of cattle, pigs and poultry[3], little work has been carried out to assess the effects of stress in transported goats especially under hot and semiarid conditions.

Stress leads to release of glucocorticoids and if prolonged may result in disease by compromising cellular and humoral immune functions, reproduction, digestion, growth and other metabolic processes[4].

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[4]. Stress in animal husbandry is related to changes in hormonal levels and blood chemistry as well as behavioral reactions. Various tressors can also activate the pituitary-adrenal axis[5].

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 adreno-medullary axis

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

Methodology	Parameters	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	holesterol esterase/Cholesterol oxidase with production of peroxide. Peroxidase addition to produce quinoneimine.	Abs(520)
Enzymatic	Urea	Urease addition 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) inpresenceof peroxidase to form a quinonimine	Abs(520)

and resulted in release of catecholamines which mobilize for short response to metabolic adjustments[5]. The other is the general adaptive syndrome describe by Selye[6] involves the activation of the pituitary-adrenal cortex resulted in release of corticosteroids which in turn extend the metabolic effects of catecholamines and adredrocorticotropic hormone (ACTH). 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 goat. 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[7].

MATERIALS AND METHODS

Animals: Thirty seven native Omani male goats ranging in body weight between 30-33 kg were used in this study. Ten goats were chosen at 6 months of age and the rest at 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 Ivomec as described by the manufacturer 0.5 mL 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 goats from each age group were divided randomly into control and stress groups. At 6 months, the 10 goats were divided equally into control and stress groups, while at 12 months the 27 goats were divided into 14 controls and 13 experiment. Three days prior to slaughter, the control goats from each age group were transported in the morning (07:00) on smooth roads in a open truck (3X2 m) at ambient temperature of 30-31°C for two h with several stop signs. They were then kept in a pen under shade in a lairage (10X10 m) at the Central Slaughterhouse at Baucher. Feed and water were available throughout the 3-day waiting period. The experimental goats 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 vacutainer (Beckton Dickson) tubes containing sodium heparin for all the treatment parameters except catecholamines where EDTA tubes were used. Blood was collected within one min from each animal with minimum disturbance to avoid excessive stress. Blood samples were kept in ice and plasma was separated within 2 of collection by centrifugation at 5°C for 10 min at 3000 rpm. The plasma was then dispensed into 1.5 mL 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), 75 mg of acid washed alumina was placed into a 2.0 mL Eppendorf tube and then 750 μL of extraction buffer, 750 μL of plasma and 100 μL of dihydroxybenzoic acid (DHBA) standard 12 ng mL^{-1} were added. This mixture was shaken for 20 min using an autovortex and then centrifuged at 5500 rpm (ALC International microcentrifuge model No. 4214) for 3 min and then the supernatant aspirated. The resulting pellet was washed with 1 mL washing buffer. The mixture was then shaken as before, centrifuged for 3 min 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 min, using the autovortex, centrifuged at 11500 rpm for 5 m 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.

Table 2: Mean±SE of steroid hormones in 6 and 12month old Omani goats subjected to transportation stress

	6 Month			12 Month		
Hormone	Control	Experimental	p-value	Control	Experimental	P-value
FSH (IU L^{-1})	0.13±0.125	0.00±0.000	0.351	00.10±0.100	0.21±0.114	0.459
LH (IU L^{-1})	0.31±0.187	0.06±0.043	0.220	00.36±0.101	0.25±0.065	0.375
TSH (nmol L^{-1})	0.04±0.019	0.02±0.005	0.482	00.19±0.047	0.18±0.055	0.897
Testosterone (nmol L^{-1})	0.02±0.017	0.00±0.000	0.363	15.00±3.840	9.39±1.648	0.198
T_3 (nmol L^{-1})	7.20±0.838	6.90±0.725	0.791	07.50±0.813	8.32±0.518	0.407
T_4 (nmol L^{-1})	14.28±1.218	13.19±0.671	0.450	12.80±0.825	12.20±0.602	0.569

Table 3: Mean±SE of serum chemistry values in 6 and 12-month old Omani goats subjected to transportation stress

	6 month			12 month		
Home one	Control	Experimental	p-value	Control	Experimental	p-value
Cholesterol (IU L^{-1})	1.91±0.128	1.66±0.051	0.103	1.81±0.155	1.71±0.095	0.579
BUN3 (IU L^{-1})	7.16±0.596	4.02±0.354	0.001	7.02±0.415	6.22±0.444	0.202
Lactate (nmol L^{-1})	2.95±0.445	4.53±0.407	0.026	2.31±0.342	6.30±1.015	0.002
Co_2 (nmol L^{-1})	19.29±0.606	22.80±0.200	0.001	24.10±0.348	23.7±0.518	0.543
Mg (nmol L^{-1})	0.90±0.015	0.97±0.036	0.121	1.02±0.0449	1.00±0.023	0.744
Po_4 (nmol L^{-1})	3.19±0.290	2.76±0.192	0.250	2.32±0.101	2.66±0.183	0.112
Ca (nmol L^{-1})	2.11± 0.037	2.07±0.058	0.561	2.60±0.048	2.60±0.072	0.961
Uric acid	0.02± 0.001	0.02±0.002	0.822	0.02±0.001	0.02±1.001	0.582
Total protein (nmol L^{-1})	57.14±1.696	51.60±2.315	0.090	80.60±0.024	82.14±1.441	0.392

Statistical analysis: The differences in the levels of the various hormones released by the control and the experimental goats were contrasted using a two way analysis of variance (ANOVA). Other comparisons between results obtained within the two age groups of goats 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[8].

RESULTS

In both age groups, the plasma concentrations of Adrenaline (AD), NorAdrenaline (NAD) and dopamine (DOP) were not significantly different ($p>0.05$) between the experimental and control goats (Fig. 1). While there was no change in cortisol level between the experimental and control 6 month old goats, in the 12 month goats, the experimental group had significantly lower cortisol than the control ($p<0.05$). Follicle Stimulating Hormone (FSH), luteinizing hormone (LH), Thyroid Stimulating Hormone (TSH), Thyroid Hormones (T_3 and T_4) and Testosterone (T) values were also not significantly different when experimental goats were compared with control group (Table 2). Equally no significant differences were found between the experimental and control goats in both age groups f or the plasma parameters (cholesterol, total protein, ammonia, uear, uric acid, Mg^{++}, Po_4^{--} and Ca^{++}), ($p>0.05$). However, there were significant differences between the experimental and control goats for blood urea nitrogen (BUN), lactate ($p<0.05$) and CO_2 ($p<0.01$). In the 6 month goats, their mean values were significantly lower in the experimental than in the control group. In the 12 month goats, only plasma lactate was significantly low in the

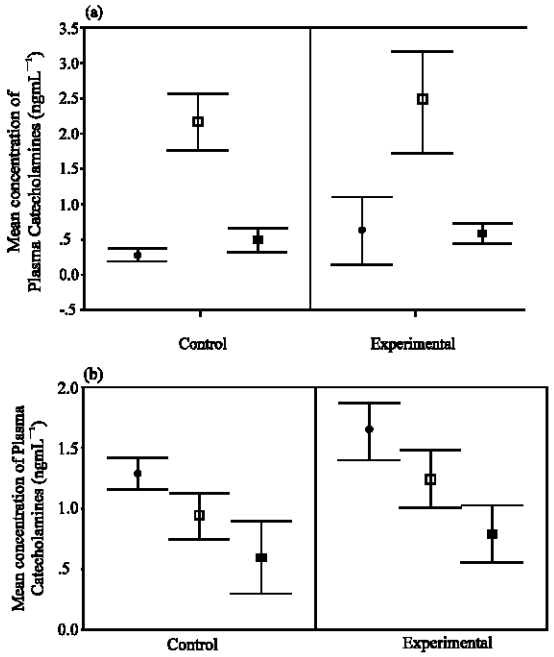

Fig. 1: Plasma catecholamine levels in Omani goats (Mean±SE) under two different treatments (experimental vs. control); Control: goats of both age groups were transported 72 h prior to slaughter; Experimental: goats of both age groups were transported at the same slaughtered day; (a) 6 month goats, (b) 12 month goats; •, noradrenaline, □ : adrenaline, ■ : dopamine; 6 month controls N=5, 6 month experimental N=5 12 month controls N=13, 12 month experimental N=14

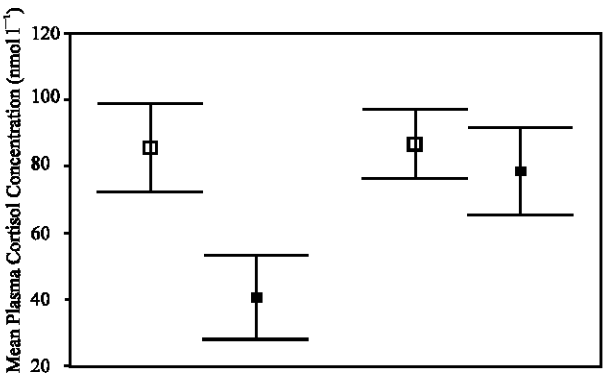

Fig. 2: Plasma cortisol levels in Omani goats (Mean±SE error) under two different treatments (experimental vs. control); Control: goats of both age groups were transported 72 h prior to slaughter; Experiential: goats of both age groups were transported at the same slaughtered day □ : 6 month goats, ■ = 12 month goats

experimental goats compared to the control group (Table 3).

Although most of the results indicated a lack of significance differences between the experimental and the control groups, the experimental group exhibited low values of some hormones and plasma parameters compared to the control groups (Tables 2 and 3). In the 6 month experimental goats the hormone values showed a drop in FSH, LH, TSH, T_3, T_4 and T relative to the control group. By contrast, in the 12-month experimental goats, the hormone values for LH, TSH, T_4 and T showed a rise over the control (Table 2). In the 6 month experimental goats, the values of the plasma parameters also dropped for cholesterol, ammonia, Po_4^{-2} and Ca^{++} and total protein. The value in experimental goats for Mg^{++} was slightly higher, while there was virtually no change in the value of uric acid. In the 12 month experimental goats there were slight increases in Po_4 and total protein and a drop in BUN3. There were no changes either in cholesterol levels, CO_2, Ca^{++}, or uric acid (Table 3).

DISCUSSION

The results of the present study indicate that short duration stressors such as transporting the goats for 2 h on an open truck, loading, unloading and blood sampling did not generate significant difference between the experimental and control groups in six and 12 month of both age goats. The catecholamines and cortisol levels, which are the major stress hormones, did not reveal any significant changes in both age groups. However, there was a slight rise in the hormonal levels in the experimental groups. On the other hand, adult sheep subjected to psychological isolation or physical stressor such as transport simulation, standing in water or handling showed an increase in cortisol and adrenaline concentrations with no effect on noradrenaline[9]. Isolation has also been shown to induce noradrenaline release[10].

The plasma cortisol, adrenaline and noradrenaline values in sheep[9] appears to be in close range to the data obtained in the present study. This may indicate that the Omani goats were either under several continuous stress factors regardless of the treatment or they may be under one specific stress factor such as heat stress since the temperature in Oman is high during most of the year. Sudden acute heat exposure of livestock is common in Oman due to the extreme climatic conditions. For example, during the morning when the goats were slaughtered around 10:30-11:00 am. The temperature was about 37.5°C. Pituitary adrenal responses of cattle to acute or gradual thermal stress resulted in a rise in cortisol concentrations in animals exposed to extreme high or cold exposure, however, gradual exposure has less effect[5].

Physiological response caused by stress may not be limited to the changes in circulating hormone levels only, but also on behavioral adjustments[11]. The high levels of cortisol in Omani goats may also be a physiological adjustment particularly to the thermal stress. According to Horton *et al.*[12], length of transportation has more marked effects on plasma cortisol levels, which, they were higher in transported lambs during the 3 day transportation period and for 2 days immediately following transport. Similar findings of transportation effects on cortisol were observed in other species. The mean plasma cortisol levels before loading in pigs was approximately 40 nmol L^{-1} and rose immediately after start of transport to 70 nmoL; and to 87 nmol l^{-1} within 10 and 30 m, respectively[13]. They found that after unloading, the cortisol levels in pigs rapidly decreased and reached a minimum level 4 h after transport. These values of cortisol in pigs are indeed similar to the data of Omani goats.

The pituitary hormones, the thyroid hormones and the testosterone levels did not change significantly due to stress. The similarities in values of gonadotropins and testosterone may indicate that they also were under similar reproductive status. However, we can't state this with certainty for the lack of sufficient data.

Rajion *et al.*[14] also reported no effects of transportation stress on cholesterol levels in goats. The increase in plasma lactate from the 12month goats in the current study is similar to that reported for other farm

animals. there was a approximately three-fold increase in plasma lactate concentrations (from 2.49±0.69 to 6.35±3.75 nmol L^{-1}) in calves subjected to transportation stress demonstrating metabolic changes typical of physiological stress reaction[15]. Other stressors (restraining and isolation) elevated serum lactate concentrations in sheep[16]. The latter authors referred this increase in blood lactate to greater rate of production than rate of uptake by skeletal muscles. This increased lactate level may affect meat quality in these animals.

Prolonged transportation of sheep produced significant effects on blood chemistry. Plasma urea nitrogen was lower in transported lambs on d 6,7 and 11 compared to controls. Plasma glucose on day four and five were lower in transported lambs and remained lower on day six and seven[12].

Although the data from this study did not reveal any significant changes in hormonal and blood parameter levels between experimental (experimental) and control groups, these results are of value for future research. Extensive experimentation on stress in Omani goats 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 goats must involve hormonal levels, plasma parameters, heart and respiratory rates, body temperature must be monitored under different sets of heat exposures. This line of study will be expanded in future since such study has an economical implication related to quality of Omani goats.

REFERENCES

1. Scharma, J.W., W. Van Der Hel, A.M. Henken, J. Gorssen and M.W.A. Verstefen, 1994. Transport of farm animals: the thermal environment. 40th ICoMST, The Hague, Hetherlands, pp: 85-96.
2. Fraser,A.F., and D.M. Broom,1990. Farm animal behaviour and welfare. 3rd Edn., CAB International, Wallingford, UK. pp: 000-000.
3. Rollin, B.E., 1995. Farm Animal Welfare. Iowa State University Press, Ames Iowa, USA. pp: 000-000.
4. Nelson, R.J. and D.L. Drazen, 2001. Seasonal Changes in Stress Reponses. In: Encyclopedia of Stress, Volume 3. Academic Press. USA.
5. Dentzer, R. and P. Mormede, 1983. Stress in Farm Animals: A bneed for re-evaluation. J. Anim. Sci., 57: 6-18.
6. Selye, H., 1936. A syndrome produced by diverse nocuous agents. Nature, 138: 32-35.
7. Broom, D.M. and K.G. Johnson, 1993. Stress and animal welfare. Chapman and Hall, London, UK.
8. SPSS., 1997. SPSS for windows. Release 7.5.2. (May 16, 1997), (SPSS Inc., Chicago).
9. Parrott, R.F., D.L. Misson and C.F. Dela Riva, 1994. Differential stressor effects on the concentrations of cortisol, prolactin and catecholamines in the blood of sheep. Res. Vet. Sci., 56: 234-239.
10. Houpt, K. A., K.M. Kendrick, R.F. Parrott, De C.F. Lariva, 1988. Catecholamine content of plasma and saliva in sheep exposed to psychological stress. Hormone and metabolic Res., 20: 189-190.
11. Leshner, A.I., 1978. An Introduction to Behavioural Endocrinology. Oxford University Press, New York.
12. Horton, G.M.J., J.A. Baldwin, S.M. Emanuele, J.E. Wohlt, L.R. McDowell, 1994. Realimentation in lambs following transport and fasting. Proceeding of the Intl. Conf. Livestock Prod. Hot Climates. Sultan Qaboos University, Muscat, Sultanate of Oman. pp: 000-000.
13. Dalin A.M., U. Magnusson, J. Haggendal, L. Nyberg, 1993. The effect of transport stress on plasma levels of catecholamines, cortisol, corticosteroid-binding globulin, blood cell count and lymphocyte proliferation in pigs. Acta Vet. Scand. 34: A59-68.
14. Rajion, M.A., S.I. Mohamed, I. Zulkifli, and Y.M. Goh, 2001. The effects or road transportation on some physiological stress measures in goats. Asian Australasian J. Anim. Sci., 14: 1250-1252.
15. Mudron P., G. Kovac, P. Bartko, J. Choma, I. Zezula, 1996. The effect of vitamin E on cortisol and lactate levels and on the acid-base equilibrium in calves exposed to transportation stress. Vet. Medicine, 41: 71-6.
16. Apple, J.K., M.E. Dikeman, Monton, J.E McMurphy, R.M. Fedde, M.R. Leith and D.E. Unruh, 1995. Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen, metabolism and incidence of dark cutting longissimus muscle of sheep. J. Anim. Sci. 73: 2295-2307.