

The influence of transportation stress on serum cortisol, thyroid hormones, and some serum biochemical parameters in Iranian cashmere (Raini) goat

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ABSTRACT

In this study the effect of transportation stress on serum concentrations of cortisol, thyroid hormones, β -hydroxybutyrate, nonesterified fatty acids, glucose and plasma total protein in Iranian cashmere (Raini) goats were evaluated. On the first day, blood samples from 10 Raini goats (5 males and 5 non-lactating non-pregnant females) were collected at 8 A.M. (T1), and after 3h food and water deprivation (T2). On the second day, samples were collected at 8 A.M. (T3), and after 3h transport (T4). Final blood sample was taken 24 h after transport ended (T5). Food and water deprivation caused a significant decrement in serum cortisol, and a significant increase between T2 and T3 was evident. Transportation caused a significant increase in serum cortisol, and the increase continued until T5. Serum T₃ showed a marginally significant decrease due to the food and water deprivation. Serum T₄ and NEFA significantly increased between T2 and T3. No significant change in serum concentrations of glucose, fT₃, fT₄, T₃/T₄ ratio and BHBA in different samplings was observed. The current study showed that short road transport had a significant effect on some stress biomarkers in cashmere goats, however, it takes a longer time before hormonal control of metabolism can be affected.

Key words: transportation stress, cashmere goat, cortisol, thyroid hormones, serum, biochemical parameters

Introduction

Endocrine response is a component of the stress response and cortisol is regarded as an indicator of stress in different species (MOHAMED, 2006; SORIANO-RODRIGUEZ et

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al., 2010). Thyroid hormones are known as important modulators of general metabolism (KANEKO et al., 2008). Thyroid hormones regulate energy metabolism and stress can affect hormonal control of metabolism (ESHRAKHAH et al., 2009). Changes in serum concentration of thyroid hormones, as important modulators of general metabolism, following environmental stresses have been reported in some domestic animals (SAEB et al., 2010), which can explain the probable correlation between serum concentrations of cortisol and thyroid hormones.

Transportation of live animals, as an inevitable husbandry practice, has been recognized as one of the main causes of stress (SAEB et al., 2010). Handling, loading, fasting, confinement, vibrations, centrifugal forces, rapidly changing light conditions, poor air quality and mixing of unfamiliar groups are some of the potential stressors during transport (SAEB et al., 2010; ZHONG et al., 2011). Transportation stress has considerable physiological effects such as increased adrenal cortical activity, decreased immunity, increased morbidity and mortality due to infectious diseases, decrease in meat quality and weight loss (SAEB et al., 2010; MAEJIMA et al., 2005). As a result, transportation stress has both economic and animal welfare concerns, and its decrement, in order to fewer side effects, has attracted considerable attention in recent years.

Transportation, as a stressful condition, activates sympathetic nervous system and hypothalamo-pituitary- adrenal axis, which is known as stress response, and results in an increment in serum concentration of cortisol (MAEJIMA et al., 2005). Cortisol is synthesized in the adrenal cortex and is regarded as an indicator of stress in different species (OKEUDO and MOSS, 2005; MOHAMED, 2006). Blood cortisol concentration is a well-known index of the reaction of animals to any environmental stressors and higher cortisol concentration may be required to meet the energy crisis during physical stress to the animals. It is believed that there is a positive correlation between the magnitude of stress challenge and the magnitude of change in metabolism (SAEB et al., 2010).

Although clinical, biochemical, hormonal, and immunological effects of transportation stress in farm animals have been evaluated, it is well established that the different animal species and even different breeds may have different responses to the same stressor (SAEB et al., 2010). On the other hand, some authors believe that different sexes may respond differently to the same stressor (BARAKA, 2012). While the effect of age on the response of animals to the stress has been documented (KANNAN et al., 2003; ZHONG et al., 2011), there is little information regarding the effect of sex on the abilities of animals to cope with transportation stress.

Goats, as economically important producers of meat, hair, and milk, have a high economic value in many countries. The Raini goat, as one of the famous cashmere breeds in Iran, is raised in large numbers in the Kerman province of Iran where goat production contributes significantly to the agricultural economy. There is little information regarding

the Raini goat in general and to the best of our knowledge, there is no previous study regarding the physiological effects of transportation stress in this valuable breed. This study was undertaken to evaluate the influence of this stress on serum concentrations of cortisol, thyroid hormones and some serum biochemical parameters, and the probable effect of sex in this breed.

Materials and methods

This study was performed in May 2014 on 10 clinically healthy pure Raini goats (5 male and 5 non-lactating non-pregnant female goats, 1-3 years old, 20-40 Kg body weight), they were selected randomly from the research farm of the Agriculture School of Shahid Bahonar University of Kerman, central Iran. The animals were reared under the same husbandry conditions in the same group pen and with free access to food (a constant diet containing alfalfa and barley) and water.

On the first day, a blood sample was collected at 8 A.M. (T1), and after 3h food and water deprivation, at 11 A.M (T2). On the second day, samples were collected at 8 A.M. (T3), and after 3h transport (T4). Final blood sample was taken 24 h after arrival (T5). Jugular blood samples were drawn in tubes containing EDTA and plane tubes, free from anticoagulant. Samples were collected by the same individual and all efforts were made to avoid exciting the animals.

The transportation of the animals was continuous and conducted at a speed of 55-65 km/h on smooth road (about 180 km), without abrupt acceleration and deceleration. The goats were transported for 3h in a closed truck, and were in standing position, with no feed and water available during transportation. Environmental temperature ranged from 20 to 25°C, and the average humidity was 25%.

The blood serum and plasma were separated after centrifugation at 750 g for 15 min, and were stored at -20 °C until analysis.

Microplate enzyme immunoassay method (Monobind Inc, Lake Forest, USA) was used for measurement of triiodothyronine (T_3), thyroxine (T_4), fT_3 and fT_4 and cortisol concentrations in serum samples. Plasma total protein was measured by refractometry method, and serum concentrations of β -hydroxybutyrate (BHBA) and nonesterified fatty acids (NEFA) were measured by kinetic enzymatic and colorimetric methods, respectively, using β -hydroxybutyrate and NEFA kits (Randox Laboratories, Crumlin, Antrim, UK). Serum glucose was measured by glucose oxidase method. The sensitivity of the cortisol, T_4 , T_3 , fT_3 and fT_4 assay was 0.366 $\mu\text{g/dL}$, 0.128 $\mu\text{g/dL}$, 0.04 ng/mL, 0.835 pg/mL and 0.314 ng/mL, respectively.

Statistical analysis was performed using SPSS18 (Illinois, Chicago). Two way repeated-measures analysis of variance tests were used to compare the serum concentration

of the measured factors between the two sexes and between different sampling periods. Correlations were analyzed by Pearson's correlation tests. Differences were considered significant at $P < 0.05$.

Results

The results of the measurement of the serum concentrations of cortisol, thyroid hormones, total protein, BHBA and NEFA in Raini goats are shown in Table 1. There was no significant difference between the male and female goats in the measured serum parameters and time x gender interaction was not significant for any parameter.

Plasma total protein significantly increased due to food and water deprivation on the first day ($P < 0.001$). Decrement was observed at T3, increased due to transportation, and decreased at T5, however, the changes were not statistically significant.

Food and water deprivation caused a significant decrement in serum cortisol on the first day ($P < 0.001$), and a significant increase between T2 and T3 ($P < 0.001$). Transportation caused a significant increase in serum cortisol (by approximately 100%, $P < 0.001$), and an increase after 24h sampling was evident ($P = 0.07$).

Serum T_3 had a decrease following food and water deprivation on the first day ($P = 0.07$). Transportation also caused a decrease in serum T_3 , which was not statistically significant. An insignificant change in serum T_4 and NEFA due to the food and water deprivation occurred. Their serum concentration increased until T3 and caused significant differences between T2 and T3 ($P = 0.045$ and $P = 0.025$, respectively). A decrease in serum T_4 and NEFA occurred during transportation, however, the differences were not statistically significant. No significant change in serum concentrations of glucose, fT_3 , fT_4 , T_3/T_4 ratio and BHBA in different samplings was observed.

There were significant correlations between serum T3 and NEFA ($r = 0.423$, $P = 0.002$), T4 and BHBA ($r = 0.483$, $P < 0.001$), and between NEFA and BHBA ($r = 0.376$, $P = 0.007$).

Table 1. The concentrations (Mean \pm SEM) of serum cortisol, thyroid hormones, glucose, β -hydroxybutyrate and nonesterified fatty acids, and plasma total protein in cashmere goats

Parameter	Sex	Time				
		T1	T2	T3	T4	T5
Cortisol (g/dL)	Male goats	27.44 \pm 3.81	10.50 \pm 0.86	11.44 \pm 0.85	25.16 \pm 3.15	29.64 \pm 2.38
	Female goats	31.66 \pm 2.71	12.30 \pm 1.26	14.16 \pm 1.45	24.16 \pm 1.92	28.64 \pm 3.65
	All sampled goats	29.55 \pm 2.3	11.40* \pm 0.78	12.80* \pm 0.91	24.66* \pm 1.74	29.14** \pm 2.06
T ₃ (nmol/L)	Male goats	4.37 \pm 0.17	4.38 \pm 0.19	4.65 \pm 0.86	4.69 \pm 0.89	4.61 \pm 0.14
	Female goats	4.41 \pm 0.18	4.38 \pm 0.11	4.70 \pm 0.23	4.22 \pm 0.17	4.71 \pm 0.22
	All sampled goats	4.39 \pm 0.12	4.38** \pm 0.11	4.68 \pm 0.11	4.46 \pm 0.12	4.66 \pm 0.12
T ₄ (nmol/L)	Male goats	63.75 \pm 5.01	68.82 \pm 5.46	87.33 \pm 4.92	76.88 \pm 4.65	80.01 \pm 5.42
	Female goats	78.11 \pm 2.55	77.01 \pm 5.37	78.96 \pm 4.22	79.74 \pm 6.57	85.20 \pm 5.23
	All sampled goats	70.93 \pm 3.57	72.92 \pm 3.86	83.15* \pm 3.66	78.31 \pm 3.83	82.60 \pm 3.65
fT ₃ (nmol/L)	Male goats	9.58 \pm 0.42	9.39 \pm 0.41	9.05 \pm 0.39	8.84 \pm 0.25	8.97 \pm 0.31
	Female goats	8.95 \pm 0.29	9.11 \pm 0.32	9.56 \pm 0.33	9.68 \pm 0.31	9.38 \pm 0.44
	All sampled goats	9.27 \pm 0.26	9.25 \pm 0.25	9.31 \pm 0.25	9.26 \pm 0.23	9.18 \pm 0.26
fT ₄ (nmol/L)	Male goats	19.77 \pm 0.54	19.33 \pm 0.88	19.09 \pm 0.67	19.36 \pm 0.42	19.53 \pm 0.67
	Female goats	19.18 \pm 0.82	19.56 \pm 0.56	15.85 \pm 0.82	19.62 \pm 0.94	19.45 \pm 0.80
	All sampled goats	19.48 \pm 0.47	19.45 \pm 0.49	17.47 \pm 0.53	19.49 \pm 0.48	19.49 \pm 0.49
β -hydroxybutyrate (mmol/L)	Male goats	0.62 \pm 0.04	0.67 \pm 0.05	0.74 \pm 0.07	0.61 \pm 0.05	0.67 \pm 0.04
	Female goats	0.66 \pm 0.07	0.66 \pm 0.07	0.78 \pm 0.06	0.76 \pm 0.05	0.77 \pm 0.07
	All sampled goats	0.64 \pm 0.04	0.66 \pm 0.04	0.76 \pm 0.04	0.69 \pm 0.04	0.72 \pm 0.04
Nonesterified fatty acids (mmol/L)	Male goats	0.16 \pm 0.01	0.15 \pm 0.01	0.18 \pm 0.01	0.17 \pm 0.01	0.16 \pm 0.01
	Female goats	0.14 \pm 0.01	0.15 \pm 0.02	0.19 \pm 0.02	0.16 \pm 0.02	0.20 \pm 0.01
	All sampled goats	0.15 \pm 0.01	0.15 \pm 0.01	0.18* \pm 0.01	0.16 \pm 0.01	0.18 \pm 0.01
Plasma total protein (g/dL)	Male goats	8.56 \pm 0.37	8.70 \pm 0.52	8.12 \pm 0.57	7.64 \pm 0.53	7.84 \pm 0.13
	Female goats	8.60 \pm 0.50	9.08 \pm 0.27	7.96 \pm 0.52	8.10 \pm 0.46	7.60 \pm 0.25
	All sampled goats	8.58 \pm 0.29	8.89* \pm 0.28	8.04 \pm 0.37	7.87 \pm 0.34	7.72 \pm 0.14
Glucose (mmol/L)	Male goats	2.05 \pm 0.10	2.13 \pm 0.067	2.17 \pm 0.083	2.12 \pm 0.090	2.23 \pm 0.075
	Female goats	2.17 \pm 0.078	2.09 \pm 0.090	2.09 \pm 0.098	2.09 \pm 0.071	2.01 \pm 0.052
	All sampled goats	2.11 \pm 0.063	2.11 \pm 0.054	2.13 \pm 0.061	2.10 \pm 0.055	2.12 \pm 0.057

T1: before food and water deprivation at first day, T2: 3h food and water deprivation at first day, T3: before transportation at second day, T4: 3h after transportation at second day, T5: 24 h after arrival, * Significant change in comparison to previous sampling (P<0.05) ** (P = 0.07)

Discussion

Although substantial study exists regarding the effects of transportation and handling stress on cattle, horse, pig, sheep, alpaca, camel and various wildlife species, there are few previous studies about the transportation stress in some breeds of goat (RAJION et al., 2001). On the other hand, there is high variability in the haematological and serum biochemical parameters between different breeds of goats (WAZIRI et al., 2010). To the best of our knowledge, no previous study exists regarding the effects of transportation stress on cashmere goats.

According to our result, a decrease in serum cortisol was evident after 3h deprivation of food and water. Similarly, deprivation of food and water for 5h in camel caused serum cortisol decrement and a circadian variation in cortisol concentrations has been proposed as the probable cause (SAEB et al., 2010). A diurnal rhythm for serum cortisol has also been documented in goat. However, some authors believed that feed deprivation stress increase the serum cortisol (KANNAN et al., 2000). In sheep, cattle and pig, feed deprivation (for 12 h, 30 h and 48 h, respectively) elevated circulating cortisol (MURAYAMA et al., 1986; WARD et al., 1992; BECKER et al., 1992). It has been reported that serum cortisol in goat peaked after feeding, while in sheep the highest values were just before feeding (ERIKSSON and TERAIVAINEN, 1989). On the other hand, 3 h feed deprivation may not be a sufficient reason for stress in goats and the changes in the following day might be due to the transportation stress.

As in our results, increase in serum cortisol due to transportation stress has been seen in previous studies in goat and also in other species, however, there is little information about the serum cortisol changes after arrival (KANNAN et al., 2000). The current study showed that 3h transportation caused serum cortisol to increase by 100% and the increment continued for the next 24h. MAEJIMA et al. (2005) reported that 1h transportation caused nine-fold increase in serum cortisol in goats. In contrast to our results, serum cortisol in camels had decreased 24h after transport termination (SAEB et al., 2010) and KANNAN et al. (2000) reported that serum cortisol in goats began to decrease 1h after transportation. Noticeable difference between different species in physiological changes due to transportation stress has been proven in previous studies (SAEB et al., 2010), and different transportation conditions can be proposed as the probable cause. It has been proven that some factors such as noise exposure and providing sufficient space to lie down, affect serum cortisol changes during transportation (KANNAN et al., 2000; KRAWCZEL et al., 2007). On the other hand, the rate of decline in serum cortisol following peak concentrations in goats varies according to the type and duration of stressors (SANHOURI et al., 1991).

Some authors believed that male animals are more excitable and stress sensitive than female animals and may have higher serum concentrations of cortisol in the same conditions (OKEUDO and MOSS, 2005). However, there were few previous studies about

the physiological response to transport stress in different sexes. The current study showed no significant difference between two sexes in serum concentration of cortisol. The same results have been reported in camel (SAEB et al., 2010). However, BARAKA (2012) reported higher serum cortisol in female diseased camels than males. Similarly, we found no effect of sex on serum concentrations of thyroid hormones in goat, which was the same as in camel during transportation stress (SAEB et al., 2010).

Endocrine response is a component of the stress response, and stress can affect hormonal control of metabolism. Thyroid hormones are known as important modulators of general metabolism, and it is believed that the evaluation of their blood concentrations has been used as an indicator of stress response (SAEB et al., 2010). There are few previous studies regarding the effects of transportation stress on serum thyroid hormones. Change in serum thyroid hormones (both T_3 and T_4) following transport stress in cattle and horse and after thermal stress in one-humped camel have been reported (NAZIFI et al., 1999; SAEB et al., 2010), which suggests the probable effect of stress on thyroid hormones. SAEB et al. (2010) found that serum T_3 had no significant change in camels and T_4 increased after 1h transportation, while T_3 and T_4 showed a significant increase after 5h transport. Long distance road transport also increased total and free iodothyronine concentrations in cattle and calves (FAZIO et al., 2001; FAZIO et al., 2005). Our results showed no significant change due to 3h transportation in serum concentrations of T_3 , T_4 , fT_3 , fT_4 and T_3/T_4 ratio in cashmere goats. Similarly, KANNAN et al. (2000) reported that 2h transportation had no effect on serum T_3 and T_4 in Alpine goats. Besides probable interspecies difference, it seems that change in blood level of thyroid hormones occurs in long distance transportation and due to long-time stressors. On the other hand, in the current study, the effect of food and water deprivation on measured serum parameters was distinguishable from that of transportation stress. Change in plasma concentrations of thyroid hormones due to prolonged food deprivation has been documented in goat (KANNAN et al., 2003). According to our results, three hours food and water deprivation resulted in serum T_3 decrease and delayed serum T_4 increase, while the changes due to the transportation stress were not significant. Neutralizing the effect of starvation by that of cortisol elevation can be proposed as a probable cause of insignificant change in serum thyroid hormones due to the transportation stress.

Lipolytic response in stressful conditions leads to mobilization of body lipid stores (SAEB et al. 2010), which may be a part of the process to meet the energy crisis during physical stress. In humans it is recognized that serum concentration of cortisol is related to the serum lipid profile (SORIANO-RODRIGUEZ et al., 2010). On the other hand, lipid metabolism can be affected by thyroid hormones, as important modulators of general metabolism, by increasing lipolysis in adipose tissue and stimulating lipogenesis by increasing the activities of some enzymes (KANEKO et al., 2008; ESHRATKHAH et al., 2009). Food deprivation and transport stress for 3h in cashmere goats did not affect the serum

NEFA. However, an increase following long-term feed deprivation in goats (KOUAKOU et al., 1999), 8h transportation in sheep (ZHONG et al., 2011) and 5h transportation in camel has been documented (SAEB et al. 2010). Although lipolysis in response to adrenal gland secretions in stressful conditions leading to mobilization of lipid stores and the current study showed an increase in serum NEFA following food and water deprivation at T3, it seems that the lipolysis response requires more than 3h. Similar to our results, SAEB et al (2010) found no change in serum BHBA due to the 5h transport of camel. BHBA is produced by NEFA being oxidized by liver, which occurs following the increment in serum NEFA and requires more time. The significant correlation between serum NEFA and BHBA, and their correlation with thyroid hormones in the current study confirmed these relationships.

Serum glucose showed no significant change due to 3h starvation and transportation stress. Similarly, ESKANDARZADEH (2014) found no significant change in serum glucose concentration due to short-term starvation in goats and believed that serum glucose in ruminant is not susceptible to short-term starvation. Transportation stress also had no effect on serum glucose in goats and calves (SANHOURI et al., 1992; SARTORELLI et al., 1992). However, an elevation in plasma glucose due to transportation in goats has been reported (KANNAN et al., 2000; RAJION et al., 2001). On the other hand, KANNAN et al. (2003) observed that glucose response to transportation stress in older goats was greater than in younger ones. The age of animals in addition to type and severity of stress can be proposed as the probable cause of the contraindications regarding the effect of stress on blood glucose in different studies.

Conclusion

The current study showed the effects of transport stress on some stress biomarkers in cashmere goats. Although 3h transportation had a significant effect on serum cortisol in cashmere goats, more time is needed before hormonal control of metabolism is affected.

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SAŽETAK

U iranskih kašmirskih (Raini) koza istražen je učinak stresa pri prijevozu na koncentraciju kortizola u serumu, hormone štitnjače, β -hidroksibutirat, neesterificirane masne kiseline, glukozu i ukupne bjelančevine plazme. Prvi dan, u 8,00 sati (T₁), uzeti su uzorci krvi od 10 Raini koza (5 mužjaka i 5 ženki koje nisu bile u laktaciji ni gravidne). Uzorci krvi ponovno su uzeti tri sata (T₂) nakon što je kozama bila uskraćena hrana i voda. Drugog dana uzorci su prikupljeni u 8,00 sati (T₃), te nakon 3 sata prijevoza (T₄). Konačni uzorak krvi uzet je 24 sata nakon što je prijevoz završio (T₅). Kortizol u serumu nakon uskraćivanja hrane i vode bio je značajno snižen, a između T₂ i T₃ značajno povišen. Prijevoz je uzrokovao značajan porast kortizola u serumu što je nastavljeno do T₅. T₃ u serumu je pokazao granično značajan pad zbog uskraćivanja hrane i vode. T₄ i NEFA u serumu bili su značajno povećani između T₂ i T₃. U različitim uzorcima nisu utvrđene značajne promjene u serumskim koncentracijama glukoze, fT₃, fT₄, omjeru T₃/T₄ i BHBA. Istraživanje je pokazalo da kratki cestovni prijevoz ima značajan učinak na neke biomarkere stresa u kašmirskih koza, međutim, za utjecaj na hormonsku kontrolu metabolizma potrebno je duže vremensko razdoblje.

Ključne riječi: stres, prijevoz, kašmirska koza, kortizol, hormoni štitnjače, serum, biokemijski pokazatelji
