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Endocrine responses to shearing stress in pregnant Ile de France ewes with low and high basal hematocrit levels

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Abstract

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The object of the present study was to investigate some hormonal responses to shearing stress. Thirty Ile De France ewes were selected from an experimental herd according to their hematocrit level and were allocated into 3 groups as follows: low hematocrit (LHct) group (hematocrit range 19.7-27.9%), high hematocrit (HHct) group (hematocrit range 32.0-36.9%) and mean hematocrit (MHct) group (hematocrit range 28.3-29.8%). The traits investigated were blood cortisol, thyroid hormones (T3 and T4), growth hormone and lactate. The experiment was conducted at the end of the first month after artificial insemination. Daily minimum and maximum ambient temperatures during the whole experimental period were 13.4 and 24.2°C, respectively. Blood samples were taken by jugular venipuncture before shearing, immediately after shearing, at 3h and 24h after shearing.

Shearing resulted in an increase in plasma cortisol in all ewes. The ewes with high baseline hematocrit level had lower percent of cortisol increase in response to shearing compared to MHct ($P < 0.05$) and LHct ewes ($P > 0.05$). Thyroxine level in LHct tended to be higher during the entire experimental period and was significantly higher compared to MHct ewes at 3 h following shearing ($P < 0.05$). Triiodothyronine in HHct ewes declined at 48 h after shearing ($P < 0.05$). There was a general trend of a slight decrease in T3 and T4 levels in all ewes at 3 h and 48 h after shearing. Blood lactate levels increased in HHct and MHct ewes at 48 h after shearing compared to the respective levels at 3 h after shearing. In conclusion, Adaptation to a lack of skin insulation at ambient temperature range 13.4 to 24.2°C was associated with a slight decrease in basal metabolism accompanied by a slight increase in lactate level.

Keywords: cortisol; growth hormone; thyroxine; triiodothyronine; lactate; stress; sheep

Introduction

Shearing of sheep is a routine management procedure in sheep breeding, which is stressful to sheep (Hargreaves & Hutson, 1990) and leads to increased production of cortisol, cytokines and malonaldehyde (Hefnawy et al., 2018). Wool removal is more stressful than any of the other manipulations involved in conventional shearing (Grandin, 2014).

Numerous studies confirm the existence of a bidirectional relationship between the endocrine system and immune function. Shearing stress is associated with increased production of cortisol and cytokines in sheep (Hefnawy et al., 2018). Thyroid hormones can also modulate innate immunity at the cellular level (Montesinos & Pellizas, 2019).

Both physical and mental stressors cause hemodynamic adjustments associated with activation of the sympathetic nervous system and hemoconcentration (Allen & Patterson, 1995). Plasma viscosity, hematocrit (Hct), red blood cells (RBC) deformability, and RBC aggregation are known to modulate blood viscosity (Nader et al., 2019). According to the optimal hematocrit hypothesis, blood viscosity increases with rising Hct levels, limiting the blood's O2 transport capacity (Schuler et al., 2010**).**

Both physical and psychological stressors are known to increase blood lactate due to stress-induced oxygen debt, determined by the high energy requirement (Hermann et al., 2019). Lactate inhibits monocyte migration and the release of cytokines TNF and IL-6 (Brooks, 2018).

In our previous study we found significant hematocrit-related difference in the magnitude of sheep adrenal response to shearing. Sheep with high hematocrit (HHct) level demonstrated more vigorous increase of plasma cortisol level in response to shearing than sheep with low hematocrit (LHct) level (Moneva et al., 2017). We reasoned that hematocrit-related specificity of adrenal response to shearing, observed in our previous experiment, is likely associated with changes in some endocrine parameters that often accompany stress.

This study was thus designed to investigate some endocrine parameters in ewes before shearing, immediately after shearing and 48 hours later.

Materials and Methods

Study site and environment data

The current study was conducted strictly in accordance with the guideline of the Institutional Animal Ethics Committee. Our investigation was carried out on May 29 and 30, 2019 at the Institute of Animal Science, Kostinbrod, Bulgaria, located at an altitude of 540 m above sea level. Mean, minimum and maximum daily temperatures during the experimental period were 19.8°C, 13.4°C and 24.2°C, respectively. Relative humidity range was 39-68%. Wind speed was in the range of 1-3 m/s.

Ewes

Institute′s research flock of 110 Ile De France ewes was used to select ewes with low, normal, and high level of hematocrit. Because of hematocrit variation, all animals were bled three times at 10-day intervals, one month before the start of the experiment in order to get correct hematocrit (Hct) values. Ewes were deprived of food the night before blood collection. In the beginning of May all ewes of the flock were artificially inseminated following estrus synchronization.

Thirty, clinically healthy Ile de France ewes were divided into 3 groups of 10 subjects each according to their hematocrit level, i.e. ewes with mean Hct level (hematocrit range of 28.3-29.8%), ewes with low Hct level (hematocrit range of 19.7-27.9%) and ewes with high hematocrit level (hematocrit range of 32.0-36.9%). The age-matched groups consisted of 3 to 5 years old ewes. During the day, the animals grazed on natural pasture and were kept in a barn at night. They received supplemental concentrate and meadow hay twice daily with free access to water.

Shearing and plasma collection

On the day of shearing, sheep were penned in a shearing shed within easy access of the shearers who removed them individually from the pen to be shorn. Sheep were shorn by professional shearers who handled the sheep in a low-stress manner.

Blood samples were collected by direct jugular venipuncture before shearing (baseline level), immediately after shearing, and at 3 h and 48 h after shearing. All blood samples were centrifuged at 5000 x g for 5 min at 10°C, aliquoted and stored at -20°C until assayed.

Estimation of blood variables

The hematological analyses were performed with whole blood samples with 5-part –differential using automated hematology analyzer (URIT-5160 Vet, URIT Medical Electronic Co., Ltd, China). The levels of hematocrit (Hct) were determined.

Blood lactate level was measured using Stat Strip Xpress lactate hospital meter (Nova Biomedical, USA) that corrects for interference due to changes in hematocrit.

Hormone measurements

Plasma cortisol, growth hormone (GH), thyroxine (T4) and triiodothyronine (T3) were estimated by commercial ELISA kits according to manufacturer‘s instructions (NovaTec Immunodiagnostica GmbH, Germany). The optical density was read at 450 nm against blank using the microplate reader (Biotek, USA).

Statistical analysis

Statistical significance was analyzed using one-way ANOVA. All data are presented as arithmetic means ± standard error of the mean (SEM). Results were considered significant when probability values (P) were less than 0.05.

Results

There was no significant difference in baseline lactate levels between the groups. Lactate levels in all the 3 groups increased immediately after shearing compared to baseline levels and recovered at 3 h after shearing (Figure 1). The ewes with low hematocrit levels showed the highest rate of lactate increase (4.08 mmol/L ± 0.29), followed by MHct (3.88 mmol/L ± 0.53) and HHct (3.37 mmol/L ± 0.36) ewes. Plasma lactate levels in MHct ewes were significantly higher at 48 h after shearing compared to LHct ewes ($P < 0.05$).

Plasma concentrations of cortisol in LHct and MHct ewes, unlike HHct ewes, were significantly higher immediately after shearing compared to baseline levels (Figure 2).

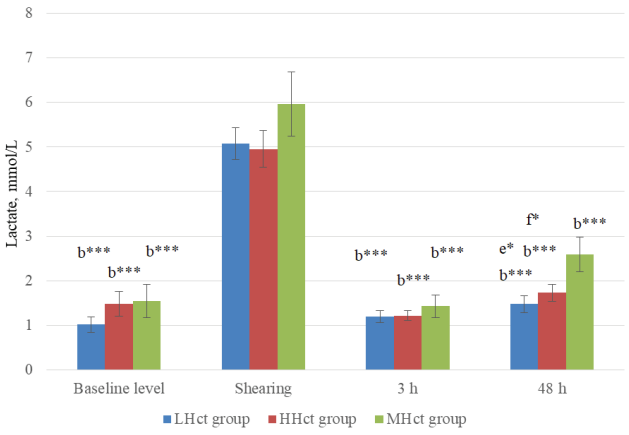

Fig. 1. Lactate levels after shearing in pregnant ewes with low and high basal hematocrit values:
b – significantly different compared to after shearing;
e – significantly different versus MHct group;
f – significantly different versus 3 h;
* – P < 0.05; *** – P < 0.001

Cortisol concentrations in HHct and MHct ewes declined significantly at 3 h after shearing (P < 0.05) compared to the concentrations immediately after shearing. In contrast to this, cortisol concentration in LHct ewes increased at 3 h after shearing (P < 0.05) and was significantly higher at that time compared to HHct ewes (P < 0.05).

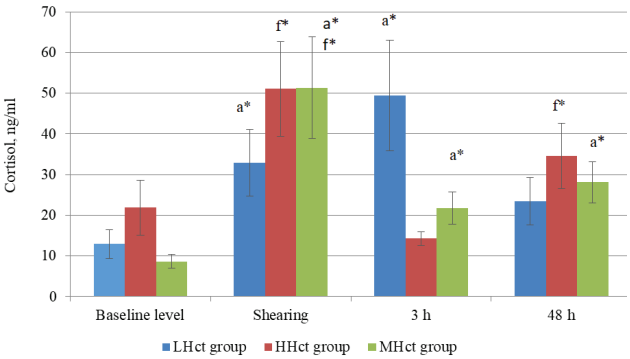

Fig. 2. Cortisol levels after shearing in pregnant ewes with low and high basal hematocrit values:
a – significantly different versus respective baseline level;
f – significantly different versus 3 h;
* – P < 0.05

Plasma GH concentration in MHct ewes increased significantly at 3 h after shearing compared to baseline level and was significantly higher at that time compared to LHct and HHct ewes (Figure 3). There was no effect of shearing stress and wool removal on plasma GH levels.

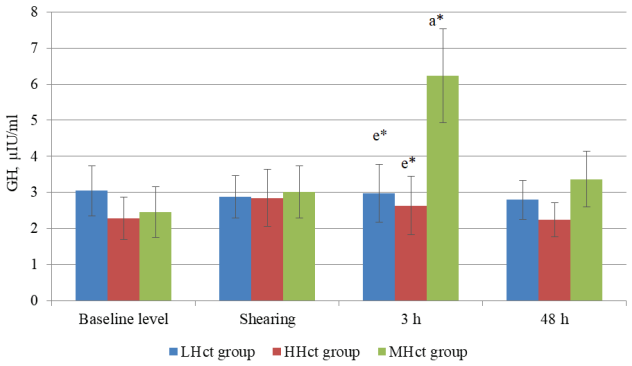

Fig. 3. Growth hormone (GH) levels after shearing in pregnant ewes with low and high basal hematocrit values:
a – significantly different versus respective baseline level;
e – significantly different versus MHct group; * – P < 0.05

There were no significant differences in baseline plasma T3 concentrations between groups (Figure 4). Plasma T3 concentrations increased insignificantly immediately after shearing in HHct and MHct ewes compared to baseline concentrations and then tended to decline at 3 h after shearing .There was significant decline of T3 concentration in LHct ewes at 3 h and 48 h after shearing compared to baseline level. Plasma T3 level in LHct and MHct ewes declined significantly at 48 h after shearing compared to the level immediately after shearing (P < 0.05).

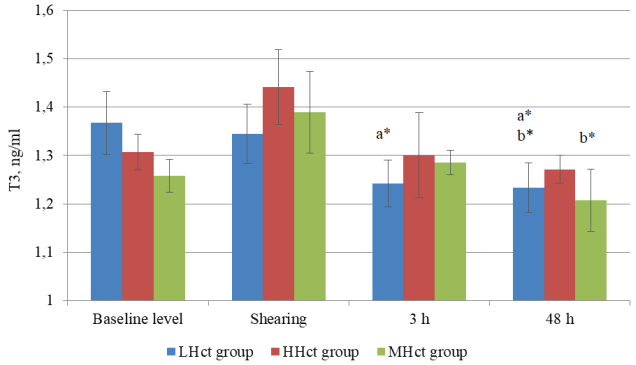

Fig. 4. Triiodothyronine (T3) levels after shearing in pregnant ewes with low and high basal hematocrit values:
a – significantly different versus respective baseline level; b – significantly different versus immediately after shearing, * – P < 0.05

Plasma T4 concentration in HHct ewes declined significantly at 3 h after shearing compared to baseline concentration (Figure 5).There was a significant decrease of plasma T4 levels in MHct ewes at 3 h and 48 h after shearing compared to baseline and immediately after shearing levels (P < 0.01).

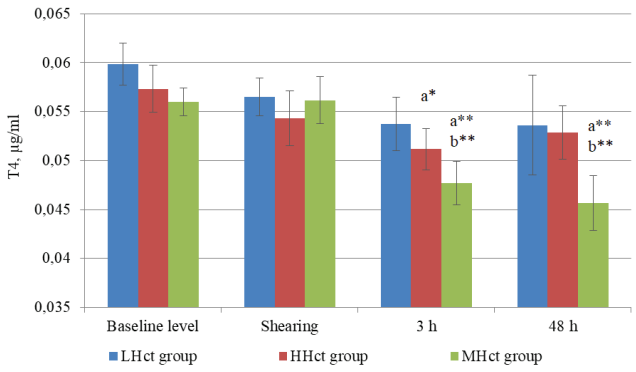

Fig. 5. Thyroxine (T4) levels after shearing in pregnant ewes with low and high basal hematocrit values:
a – significantly different versus respective baseline level;
b – significantly different versus immediately after shearing,
* – $P < 0.05$; ** – $P < 0.01$

Discussion

The increase of lactate levels in HHct and MHct ewes at 48 h after shearing compared to the levels at 3 h after shearing may reflect an attempt of the body to cope with the extra energy requirement to regulate body temperature after shearing when the animals experience some degree of cold (Figure 1). Respiration rate is one of the biomarkers of heat stress. Sheep use respiratory evaporative cooling through the process of multifold increase in respiratory rate when exposed to heat (Rashamol et al., 2018). Severity of heat stress has been proposed to be quantified according to panting rate – low: 40-60; medium high: 60-80; high: 80-120; severe above 120 breaths per min (Silanikove, 2000). Shearing narrows the limits of the thermoneutral zone and therefore can induce cold stress in the morning and heat stress in the early afternoon. The actual lower critical temperature may vary considerably depending upon age, breed type, nutrition and wool coat. Lower critical temperatures in shorn sheep under maintenance feeding and under full feed are found to be 28°C and 13°C respectively (NRC, 1981). Sheep shearing at maximum ambient temperature of 10.7-17.9°C has been reported to result in a substantial drop in respiratory frequency (under 40 breaths per min) in the morning (Aleksiev, 2008). Similar decline in respiratory frequency have been observed in dairy ewes on the first day following shearing at much higher ambient temperature – 28-29°C (Piccione et al., 2008). Given that, low critical temperature rises up to 28°C and respiratory rate declines sharply at ambient temperature of up to 28°C on day 1 after shearing, we are prone to believe that under the temperature range in our experiment (13.4 – 24.2°C) sheep experienced a mild cold stress. This assumption is further supported by the hormonal data. Glucocorticoids participate in regulation of iodine thyroid homeostasis. Stress is suggested to cause a decrease of thyroid stimulating hormone (Nadolnik, 2012). Thyroid hormones did not change significantly (Figures 4 and 5) in response to shearing in spite of the increased cortisol levels at that time (Figure 2) suggesting no effect of cortisol on thyroid function during acute stress (Figures 4 and 5)

On the other hand, the observed T3 level decline in LHct ewes at 3 h after shearing (Figure 4) coincided with a significantly higher cortisol level (Figure 2) at that time suggesting a possible association between cortisol and T3. Alternatively, the observed lower T3 level in LHct ewes at 3 h after shearing must be independent of the simultaneous activation of the adrenal cortex. It is worth noting that the difference in T4 levels between LHct and MHct ewes, although not significant, persisted at 48 h after shearing, and reflect a possible difference between the two groups in thyroid response to cold stress (Figure 5). Lactate level was significantly higher in MHct ewes compared to LHct ewes (Figure 1) at that time ($P < 0.05$). The higher lactate level in MHct ewes might be due to a lower aerobic metabolism and reduced clearance of lactate in response to cold conditions (Stainsby & Brooks, 1990). The lack of change in T4 concentration in LHct ewes during entire experimental period accompanied with a significant decline in T3 concentrations at 3 and 48 h after shearing is consistent with the reported inhibitory effect of chronic physiologic stress on deiodinase (D)1 activity (Holtorf, 2014). Therefore, the observed decline of T3 and T4 concentrations in LHct ewes may be due to reduced conversion of the inactive T4 to the active T3. Also, stress-induced increase in type 3 deiodinase shift conversion of T4 from biologically active T3 to biologically inactive reverse T3. Furthermore, inflammatory cytokines have also been found to reduce D1 activity, resulting in decreased T3 levels and increased reverse T3 levels (Mehta et al., 2016). Consequently, increased IFN-γ concentration in LHct ewes may also be associated with the observed changes of thyroid hormones in LHct ewes.

Besides, increased lactate level in HHct ewes at 48 h after shearing compared with lactate level at 3 h after shearing was accompanied by a significant decrease in plasma T3 levels at that time compared to the level after shearing. These data give further support of the assumption that lactate level increase in HHct and MHct ewes at 48 h after shearing was caused by a reduction of basal metabolic rate. Our results are consistent with previous studies showing that short-term cold air exposure (30 min to several hours) either do not cause any changes in serum levels of thyroid hormone and TSH or cause a slight

decrease (Pääkkönen & Leppäluoto, 2000) and support our assumption that shearing under the temperature range of our experiment resulted in a mild cold stress.

Growth hormone is known to stimulate sweat secretion and heat evaporation during exercise. The increase in GH release during exercise is associated with the concomitant increase in body temperature. However, exercise performed at 4 °C results in a suppression of GH secretion (Jørgensen et al., 2003). We did not find significant changes in GH levels throughout the entire experimental period except for significantly higher GH level in MHct ewes compared to LHct and HHct ewes at 3 h following shearing (Figure 3). The major findings of a recent review of the research on multiple effects of GH suggest a role of GH in the regulation of angiogenesis, immune function, hematopoietic system, normal differentiation and function of blood cells (Devesa et al., 2016). Therefore, increased GH level in MHct ewes can be associated with one or more of its many functions.

It has been shown that cortisol-dependent regulation of uteroplacental glycolysis may allow increased maternal control over fetal nutrition and metabolism during acute stress in ewes. Also, the authors found cortisol-initiated switch from supplying glucose to the fetus to a greater dependence on lactate (Vaughan et al., 2016). Therefore, increased cortisol levels in LHct ewes at 3 h after shearing compared to HHct ewes may indicate better fetal nutrient supply in response to stress-induced oxygen and energy deficit.

Overall, the results of hormonal dynamics give further support of the view that shearing of sheep at ambient temperature range from 13.4°C to 24.2°C results in a mild cold stress.

Conclusions

Sheep shearing elicited significant rise in plasma cortisol, which is widely believed to be marker of energy deficiency and inadequate oxygen delivery.

The loss of the insulation provided by wool resulted in a slight decline in basal metabolism accompanied by a slight increase in lactate level.

Acknowledgements

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