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Hematologic 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 hematological 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 lactate and hematological indices. 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 blood values of red blood cells, hemoglobin and hematocrit as well as a decrease in plasma volume in all ewes. Nucleated red blood cells (NRBCs) count and mean corpuscular hemoglobin concentration (MCHC) declined significantly in LHct ewes only. Low hematocrit ewes had higher percent of hematocrit ($P > 0.05$) and lactate ($P > 0.05$) increase in response to shearing, compared to HHct ewes. All blood parameters returned to normal values at 3 h after shearing. There was a significant correlation between the rates of NRBC and plasma volume decline ($r = -0.66779$) in response to 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.

The data reported herein suggests that LHct ewes, unlike HHct ewes have higher capacity to cope with acute stress-induced increase in oxygen and energy demand. Also, adaptation to a lack of skin insulation at ambient temperature range 13.4 to 24.2˚C was associated with reduction of lactate disposal.

Keywords: hematocrit; red blood cells, mean corpuscular hemoglobin concentration; nucleated red blood cells; 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.

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 O_2 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). To the best of our knowledge, there is no study relating inter-individual differences in sheep baseline hematocrit to hematological adjustments in response to stress.We reasoned that hematocrit-related specificity of adrenal response to shearing, observed in our previous experiment, is likely associated with changes in some hematologic parameters that often accompany stress.

This study was thus designed to investigate some hematologic 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 venepuncture 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 red blood cells (RBC),), nucleated red blood cells (NRBC), hematocrit (Hct), hemoglobin (Hgb) and mean corpuscular hemoglobin concentration (MCHC), were determined. Reticulocytes were stained with New methylene blue and counted microscopically. We followed the procedure of Briggs and Bain described by Bain et al. (2012).

Corrected reticulocyte count was calculated using the following formula:

Corrected Reticulocyte count = Reticulocyte percentage x (Hemoglobin/Normal Hemoglobin).

Changes in plasma volume after shearing were estimated by the equation of Dill &Kostill (1974) as follows:

$$BV2 = (BV1)\,(Hgb1/Hgb2)$$
$$CV2 = (BV2)\,(Hct2/100)$$
$$PV2 = BV2 - CV2$$
$$\Delta PV, \% = (100)\,(PV2 - PV1)/PV1,$$

where BV = blood volume, CV = red cell volume, PV = plasma volume, Hgb = hemoglobin, Hct = hematocrit, subscript 1 refers to baseline sample, subscript 2 refers to stressor sample, $BV1$ is taken as 100 and $PV1$ is 100 – $Hct1$.

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

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

Plasma volume decreased significantly in response to shearing. Calculated changes in plasma volume immediately after shearing relative to baseline volume in ewes with low hematocrit (LHct), mean hematocrit (MHct), and high hematocrit (HHct) baseline values were 19.8% ± 2.27; 15.60% ± 3.680, and 12.05% ± 2.31 ($P < 0.05$), respectively. No significant differences were found between the groups.

Hematocrit level in LHct ewes was significantly lower throughout the experimental period compared to HHct ewes and MHct ewes (Figure 1). Also, baseline Hct level in MHct ewes was significantly lower compared to HHct ewes. Hematocrit level in all 3 groups increased significantly immediately after shearing and then declined towards baseline level at 3 h and 48 h following shearing. The rates of hematocrit levels increase immediately after shearing was insignificantly higher in LHct ewes compared to HHct ewes (5.375% ± 0.89 versus 3.185% ± 0.84, respectively).

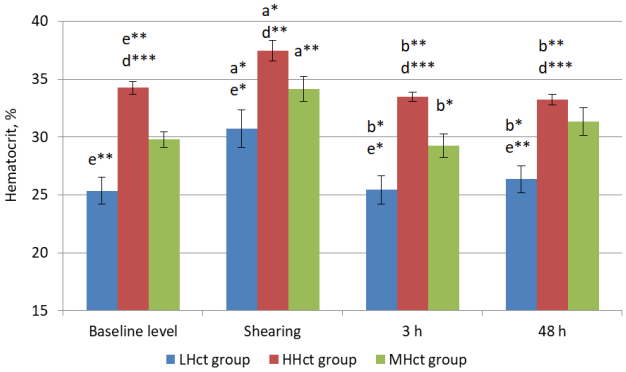

Fig. 1. Hematocrit after shearing in pregnant ewes with low and high basal hematocrit levels:
a – significantly different versus respective baseline level;
b – significantly different versus immediately after shearing;
d – significantly different versus LHct group;
e – significantly different versus MHct group;
* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$

Red blood cells and hematocrit values had similar patterns of dynamic, the only difference being that MHct ewes had significantly lower RBC counts compared to HHct ewes at 3 h following shearing (Figure 2).

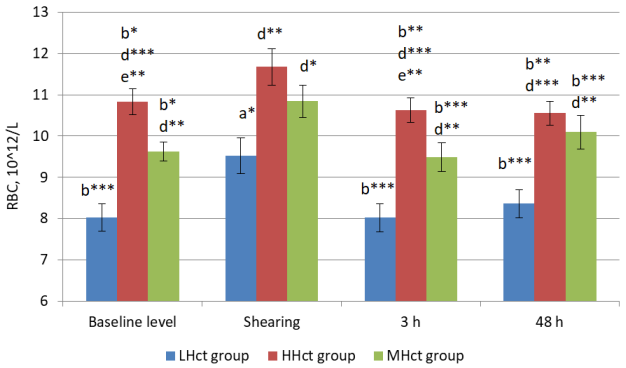

Fig. 2. RBC after shearing in pregnant ewes with low and high basal hematocrit level:
a – significantly different versus respective baseline level;
b – significantly different versus immediately after shearing;
d – significantly different versus LHct group;
e – significantly different versus MHct group;
* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$

Hemoglobin level was higher in HHct ewes among all measurements, followed by MHct and LHct ewes (Figure 3). Hemoglobin level increased significantly immediately after shearing in all the 3 groups, then declined at 3 h and remained unchanged at 48 h after shearing. However, the rate of Hgb increase was significantly higher in LHct ewes, compared to HHct ewes (8.45%).

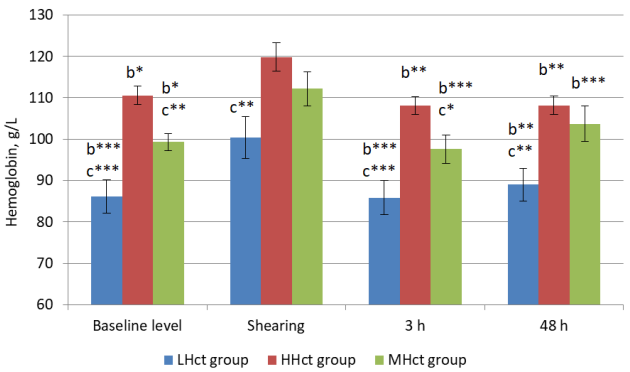

Fig. 3. Hemoglobin after shearing in pregnant ewes with low and high basal hematocrit levels:
b – significantly different versus immediately after shearing;
c – significantly different versus HHct group;
* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$

Mean corpuscular hemoglobin concentrations are presented in Figure 4. The results indicate significantly higher MCHC in LHct ewes compared to the other two groups among all measurements, except for the levels immediately after shearing and 3 h after shearing, compared to MHct

group. Values of MCHC in MHct ewes were significantly higher compared to HHct ewes at 3 h following shearing. Shearing resulted in a significant decline of MCHC in LHct ewes and a trend of decline in HHct and MHct ewes.

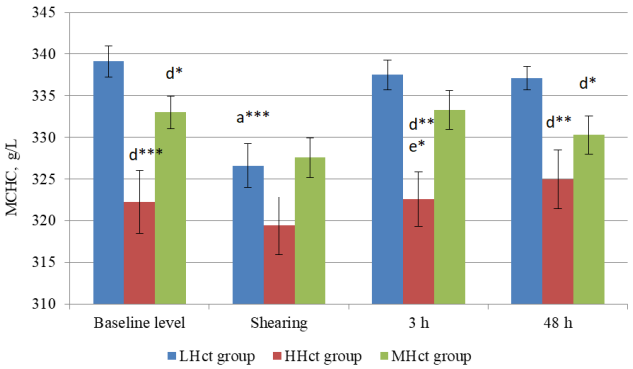

Fig. 4. MCHC after shearing in pregnant ewes with low and high basal hematocrit levels:
a – significantly different versus respective baseline level;
d – significantly different versus LHct group;
e – significantly different versus MHct group;
* – P < 0.05, ** – P < 0.01, *** – P < 0.001

The reticulocyte percentage and corrected reticulocyte count at 48 h after shearing are presented in Figure 5. The results indicate significantly higher corrected reticulocyte count in HHct ewes relative to LHct ewes (P < 0.05).

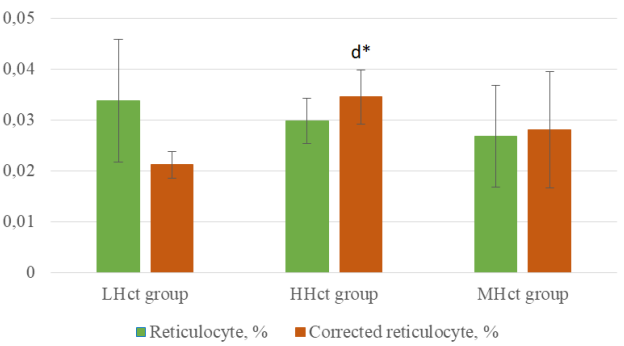

Fig. 5. Reticulocytes (%) and corrected reticulocyte count at 48 h after shearing in pregnant ewes with low and high baseline hematocrit levels:
d – significantly different versus LHct group;
* – P < 0.05

The mean percent change in the NRBC is presented in Figure 6. Nucleated red blood cell values in all the 3 groups declined immediately after shearing and returned to baseline levels at 3 h after shearing, but the differences were only significant in LHct ewes.

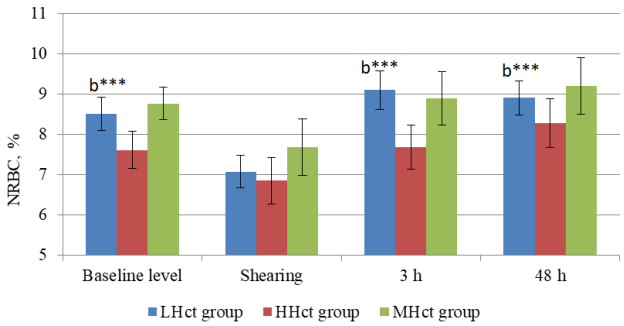

Fig. 6. NRBC after shearing in pregnant ewes with low and high basal hematocrit levels:
b – significantly different versus immediately after shearing;
*** – P < 0.001

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 (Figure7). 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).

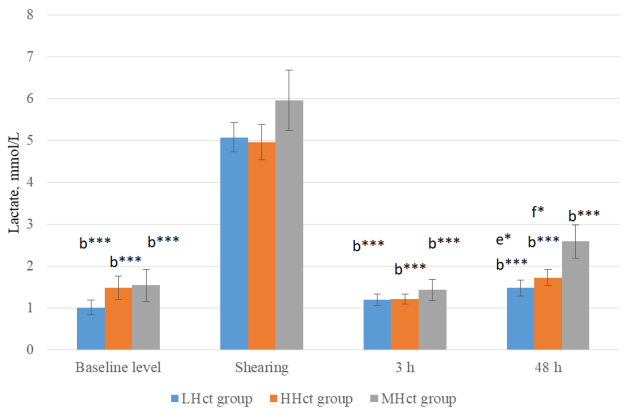

Fig. 7. Lactate after shearing in pregnant ewes with low and high basal hematocrit levels:
b – significantly different versus immediately after shearing;
e – significantly different versus MHct group;
f – significantly different versus 3 h;
* – P < 0.05; ***- P < 0.001

Discussion

Shearing elicited significant plasma volume decline in all ewes used in this study (n=30). Similar changes in plasma volume have been observed during exposure to both physical stress (Beaumontet al., 1981; Costill &Fink, 1974; Connes et al., 2013) and psychological stress (Patterson et al., 1995; Patterson et al., 1998). It is generally accepted that an important mechanism for stress-induced decreases in plasma volume is increased blood pressure leading to increased fluid shifts from the vasculature into the interstitial spaces (Patterson et al., 1995). The general response to stress is the activation of the sympathetic nervous system with inhibition of the parasympathetic nervous system (Ziegler, 2004). Stress-related increase in catecholamine levels can produce significant changes in total plasma protein, plasma viscosity, hemorheology and hemoconcentration (Patterson et al., 1998). Calculated plasma volume from hematocrit and hemoglobin is suggested to be an indirect measure of hemoconcentration (Austin et al., 2011). Shearing-induced stress in our study reliably increased the indices of hemoconcentration (hematocrit and hemoglobin). Elevated blood concentrations of hemoglobin and Hct in HHct ewes compared to the other groups suggest that they have improved oxygen transport capacity, and therefore increased amount of oxygen that can be carried for volume of blood **(**Mairbäurl, 2013). Given that, acute stress is associated with increased sympatho-adrenal activity and subsequent hemoconcentration (Muldoon et al., 1992), it can be assumed that stress-induced hematological adjustments are an integral part of the hypothalamic-pituitary-adrenal system that contribute to a faster rate in tissue and blood oxygenation (Muldoon et al., 1992). Stress is known to elicit significant increase in blood noradrenaline and adrenocorticotropin (Koelschet al., 2016). Also, heightened sympathetic arousal and increased hemoconcentration that occur in response to acute stress suggest that hemoconcentration can be used as a potential indirect marker of sympathetic activation. Increased hemoconcentration in this study is consistent with the increases of energy and oxygen demands during the classical alarm response. It is hardly surprising that Hargreavets& Hutson used hematocrit along with cortisol as markers of stress caused by shearing (Grandin, 2014).

The quick return of plasma volume to baseline values in all the 3 groups at 3 h following shearing implies that hemoconcentration was due to reversible shifts in plasma volume. It is worth noting that the rate of Hct levels increase from baseline to immediately after shearing was significantly higher in LHct than HHct ewes in spite of the higher Hct level in HHct ewes immediately after shearing (Figure 1). Similar changes were observed in hemoglobin and RBC values (Figure2). The most common assumption regarding hematocrit and hemoglobin concentrations is that increases in hematocrit and hemoglobin are associated with increased VO2 max (Jacobs et al., 2011). Therefore, it is plausible to assume that higher baseline hematocrit level observed in HHct ewes relative to LHct and MHct ewes is associated with better oxygen carrying capacity and higher oxygen requirements at rest compared to the other groups. Our assumption also suggests that shearing- induced stress is supposed to result in a higher increase of oxygen demand, and thus higher rate of hematocrit response in HHct ewes compared to LHct ewes. Contrary to our expectation, the rate of hematocrit level increase was highest in LHct ewes compared to HHct and MHct ewes. The higher rate of increase in hematocrit levels from baseline to post shearing observed in LHct ewes compared to HHct ewes suggests that the rate of sympathetic activation and adrenal secretion of catecholamines were higher in LHct ewes in spite of the significantly higher hematocrit level in HHct ewes immediately after shearing (Figure 1).

The observed discrepancy can be reasonably explained by the paradox of hematocrit in exercise physiology, where subjects with low hematocrit have also higher values of aerobic working capacity. Blood viscosity is inextricably related to oxygen delivery. There is an inverse relationship between cardiovascular fitness and hematocrit, where individuals with higher aerobic capacity display lower hematocrit measures (Brun et al., 2000). The slight decrease of hematocrit in elite athletes by training is brought about by an increase in plasma volume (Allen et al., 1992; Mairbäurl, 2013). There is scientific data that supports the fact that an increase in blood fluidity via plasma volume expansion (autohemodilution) lowers both plasma viscosity and hematocrit (Brun et al., 1989; Brun et al., 1998). Schuler et al. (2010) proposed the optimal hematocrit hypothesis. According to this hypothesis, blood viscosity increases with rising hematocrit levels, limiting the blood's oxygen capacity. Given that augmented hemoglobin values are associated with a rise in blood viscosity and, consequently, with a higher peripheral vascular resistance that may reduce VO2 max (Guyton et al., 1961), we are prone to believe that hematocrit value observed in HHct ewes immediately after shearing (37.442 ± 0.901) was close to optimal hematocrit and its further increase would impair oxygen delivery to the tissues (Soni et al., 1993). More investigations are needed to determine the exact optimal hematocrit value in this breed of sheep since blood fluidity is not only influenced by hematocrit value, but also by blood viscosity, red blood cell deformability, relative ratio of young red blood cells, ATP and NO release from red blood cells (Rozier et al., 2007; Mairbäurl, 2013).

Our assumption that LHct ewes had higher rate of sympathetic activation in response to shearing compared to the other groups is further supported by lactate data (Figure7). The percentage of lactate level increase from baseline to after shearing was insignificantly higher in LHct ewes compared to HHct ewes (4.08 mmol/L ± 0.29 versus 3.37 mmol/L ± 0.36 respectively) in spite of the similarity in lactate levels immediately after shearing. This is consistent with reported increases in epinephrine and norepinephrine concentrations in a clear association with lactate production. Also, the lactate inflection point is attributable to a sudden rise in catecholamine levels (Brooks, 1986). Furthermore, sympathetic-adrenal-medullary system activation during exercise is crucial for lactate rate of appearance (Simões et al., 2003; Kubera et al., 2012; Messonnier et al., 2013). The similarities between dynamic patterns of hematocrit and lactate in response to shearing observed in our study give further support to our view that both hematocrit and lactate increases were due to sympathetic activation and increased catecholamine secretion rate (Stainsby et al., 1984).

However, the rate of hematocrit increase in response to shearing was significantly higher in LHct compared to HHct ewes, while the rate of lactate increase did not reach level of significance. This discrepancy can be explained by the fact that lactate disposal is mainly through oxidation, especially during stress when oxidation accounts for 70-75% of removal and gluconeogenesis the remainder (Brooks, 2007). The higher rate of hematocrit increase observed in LHct ewes in response to shearing suggests a higher rate of increase in oxygen carrying capacity of blood and thus increased lactate disposal via oxidation. Given that lactate production and lactate removal occur simultaneously, it is not surprising that the difference in the rate of lactate increase between LHct and HHct ewes did not reach level of significance immediately after shearing. Hematocrit level in HHct ewes immediately after shearing was higher compared to the other 2 groups, suggesting higher arterial oxygen content (Figure1). However, it has been shown that blood viscosity increases with rising hematocrit levels limiting the blood's oxygen transport capacity (Schuler et al., 2010).

Therefore, similarities between blood lactate levels in LHct and HHct ewes immediately after shearing were most probably due to differences in the rate of lactate production and disposal. A positive relation has been found between lactate uptake by red blood cell, MCH and MCHC (Munoz et al., 1996). Lactate uptake into red blood cells has been suggested to reduce plasma lactate concentration (Mairbäurl, 2013). It is therefore expected that the observed differences in MCHC between the groups may differentially influence plasma lactate content. Furthermore, in our earlier study (unpublished data) we found insignificantly higher body weight in HHct ewes compared to the other two groups (i.e. 61.50 ± 2.910 kg; 63.675 ± 2.939 kg; 62.70 ± 2.162 kg in LHct, HHct and MHct ewes respectively). These findings are in agreement with the reported inverse relationship between body mass and velocity of lactate threshold in healthy male runners (Buresh et al., 2004) and suggest that lactate inflection point in the HHct ewes may be lower compared to the other groups. Besides, plasma cortisol levels in HHct ewes tended to be higher during the entire experimental period compared to LHct and MHct ewes (unpublished data) which may be related to a relatively higher level of lactate disposal via gluconeogenesis compared to the other groups due to glucocorticoid-stimulated gluconeogenesis (Holloszy, 1984; Sistare &Haynes, 1985). This view is further supported by the significantly higher baseline and stress-induced levels of cortisol in HHct ewes compared to LHct ewes found in our earlier study (Moneva et al., 2017). Moreover, according to the lactate shuttle concept, skeletal muscle both produces and uses lactate as a fuel with much of the lactate formed in glycolytic fibers being taken up and oxidized in adjacent oxidative fibers. This use of lactate as a fuel requires mitochondrial respiration, thus it follows that an ability to rapidly utilize lactate requires ample mitochondrial abundance and respiratory capacity (Brooks, 2002; Brooks, 2018). Therefore, a possible difference in muscle mitochondrial density among the groups may influence lactate removal. Furthermore, it has been suggested that selection for increased body weight entails higher conversion from type IIa (fast oxidoglycolytic) to type IIb (fast glycolytic muscle fibers).

Also, domestic pigs have been shown to have a high percentage of type II fiber compared to wild pigs (Ruusunen &Puolanne, 2004). Similar relation in muscle fiber types has been observed in different Japanese quails lines (Muir&Aggrey, 2003). The most pronounced shift from an oxidative to a more glycolytic metabolism has been observed in double-muscled compared to non-double muscled Belgian blue bulls. Moreover, due to the reduction of capillary density, the oxygen supply has also been reduced which may impair elimination rate of lactate (Fiems, 2012). The Ile De France sheep used in our study is one of the major meat breeds. Lambs have a high growth rate. Increased growth rate of type IIb fiber after birth compared to type I fiber is positively correlated to body weight and muscle mass (Choi&Kim, 2009).

These findings suggest that HHct ewes may have higher percentage of type IIb (glycolytic) fibers and therefore higher need of oxygen to remove increased lactate production. This suggestion is consistent with our view that higher baseline levels of Hct, Hgb and RBC in HHct ewes represent

general hematologic adjustment in response to higher oxygen demand, due to increased lactate production. Besides, in addition to the aforementioned hematological variables, the lower baseline MCHC in HHct ewes may also be considered part of the general hematologic adjustment to increased oxygen demand, due to the inverse relationship between MCHC and erythrocyte deformability (Tempelhoffet al., 2016). The high deformability of red blood cell facilitates blood flow even at high hematocrit level (El-Sayed et al., 2006).

In addition, the hematological adjustments to the assumed higher blood viscosity in HHct ewes also included increased erythropoiesis as judged by the higher corrected reticulocyte count in HHct ewes (Figure 7). The young red blood cells are characterized by improved oxygen release and deformability, both of which improve tissue oxygen supply (Mairbäurl, 2013).

Thus, increased RBC deformability and slightly higher erythropoiesis in non-stressed HHct ewes compared to LHct and MHct ewes is expected to improve blood oxygen carrying capacity. However, the capacity of HHct ewes to improve tissue oxygen supply in response to shearing-induced increase in oxygen demand was significantly reduced compared to LHct ewes as judged by the lower percentages of MCHC and plasma volume decline immediately after shearing (Figure 4). The lower rate of Hct increase in response to shearing in HHct compared to LHct ewes might be explained by the fact that an increase in hematocrit results in a linear increase in oxygen carrying capacity of blood and exponential increase in viscosity. Therefore, HHct ewes have less capacity to further increase hematocrit value in response to stress-related increase in oxygen demand. We don't know the exact optimal hematocrit value in sheep, particularly hematocrit in Ile De France breed, but it is obvious that the higher baseline Hct value in HHct ewes is closer to the optimal hematocrit level compared to LHct ewes. It must be stressed that the decline of MCHC in LHct ewes immediately after shearing was more pronounced compared to HHct and MHct ewes, suggesting better fluidity and higher tissue oxygen supply.

Taken together, these findings suggest that higher baseline levels of Hct, Hgb and RBC, and lower level of MCHC in HHct ewes compared to the other groups, represent hematological adjustments to increased oxygen demand.

Reticulocytes are generally absent in peripheral blood of healthy adult cattle and goats but a small number of punctate types (0.5%) may occur in adult sheep (Kaneko et al., 1997). Since the number of reticulocytes in the peripheral blood reflects the activity of the bone marrow, reticulocyte counting has been widely used for evaluation of hematopoiesis (Riley et al., 2001). A slight but significantly higher value of the corrected reticulocyte count was found in HHct ewes compared to LHct ewes (Figure5), suggesting higher rate of erythropoiesis. This view is consistent with the higher RBC count in HHct ewes.

The peripheral blood of healthy adults is generally free of NRBC. However, nucleated red blood cells are commonly found in neonatal blood. Fetal NRBC have been widely reported in maternal blood during pregnancy (Shulman et al., 1998). Therefore, we assume that NRBC found in maternal blood of sheep are of fetal origin. This is the first study showing that NRBC are present in maternal blood of sheep at the end of the first month of pregnancy. It has been proposed that fetal NRBCs transferred to maternal blood are subjected to apoptosis due to high oxygen concentration in maternal blood (Kondo et al., 2002). Recent studies indicate that it will soon be possible to distinguish NRBC of fetal origin from that of maternal NRBC (Oosterwijk et al., 1998; Chaet al., 2003). The observed decline of NRBC in all 3 groups in response to shearing reached level of significance in LHct ewes only (Figure 6). There was significant correlation (r=-0.66779) in the percent decline between NRBC and plasma volume immediately after shearing relative to the respective baseline values ($P < 0.001$). It is worth noting that LHct ewes had insignificantly higher percent of plasma volume decline immediately after shearing compared to the other two groups. These data suggest that shearing-induced decrease in NRBC is associated with a simultaneous decrease in plasma volume. However, plasma volume decline was expected to increase NRBC count. Instead, NRBC count declined. The mechanism responsible for this decline is not clear. Interestingly, Fig. 6 and Fig.4 indicate similar patterns and dynamics of NRBC count and MCHC. Both NRBC and MCHC declined significantly in response to shearing in LHct ewes only. There was a slight but significant correlation between MCHC and NRBC rates of decline immediately after shearing compared to baseline levels. The correlation coefficient between the two variables was 0.470 ($P < 0.05$). The observed similarity between these hematologic variables gives rise to the notion of a possible association between MCHC and NRBC appearance in peripheral blood. One possible explanation of this association is that both parameters were influenced by stress-induced hypoxia, and hypoxia has been shown to elicit hematopoietic stress (May et al., 2019).

Conclusions

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

The major hematological responses to shearing were manifested by an increase in hematocrit, red blood cells and

hemoglobin values as well as by a decrease in mean corpuscular hemoglobin concentration. These changes were more pronounced in LHct ewes, suggesting that LHct ewes have higher potential for hematologic response to acute stress, as regard their capacity to increase the rate of hemoconcentration, blood fluidity, and ultimately tissue oxygen delivery.

Adaptation to a lack of skin insulation at ambient temperature range 13.4 to 24.2˚C was associated with reduction of lactate disposal.

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