Acclimatization to moderate altitude in ewes having low or high hematocrit levels

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Abstract

The object of the present study was to investigate the acclimatization strategy to mild hypoxia in ewes having low or high hematocrit levels. Ile De France ewes were selected according to their levels of hematocrit and then were allocated into two groups. Group I comprised ewes with low hematocrit level (n-10) and group II comprised ewes with high level of hematocrit (n-10). Immediately after the shearing, performed at the experimental unit of the Institute of Animal Science, Kostinbrod, (altitude of 500 meters) the ewes were transported to a mountain pasture at altitude of 1440 m where they were raised for 4 months (June–September). Blood samples were collected via venipuncture at the following time points: before shearing, immediately after the transport, on day 5, 10, 30 and 60 after the transport. The following indices were measured: plasma cortisol, hematocrit, total leukocyte count, erythrocyte count and reticulocyte count. Adrenal response to transport and exposure to altitude was significantly higher in high-hematocrit ewes relative to low-hematocrit ewes. The higher hematocrit level in the ewes of group II persisted throughout the experiment. The ewes having high hematocrit level had higher cortisol concentration immediately after transport and during the rest of the experimental period as compared to low-hematocrit ewes. Erythrocyte count tended to be higher in high-hematocrit ewes throughout the experimental period. Reticulocyte count increased significantly as compared to basal count and remained elevated in both groups at the mountain pasture. There was distinct difference in the reticulocyte number dynamics among the groups. Leukocyte count tended to be higher in the high-hematocrit ewes immediately after transport and on day 5 and 10 following exposure to altitude. The observed trend reached significance on day 30 and 60. It was concluded that basal hematocrit level is related with the pattern of adrenal and hematological adjustments of newly shorn ewes to moderate altitude.

Key words: cortisol, hematocrit, leucocytes, reticulocytes, acclimatization, sheep

Introduction

Human populations living at high altitude have a metabolic adaptation associated with improved muscle energetics, enhanced tissue oxygen delivery, enhanced mitochondrial coupling efficiency and elevated circulating NO metabolites (Horscroft et al., 2017; Murray et al., 2018). Furthermore, they display unique hematological adaptations to life at high altitude (Bigham and Lee, 2014).

Investigations on sheep adaptation to high altitude are scarce. A relationship between blood-related phenotypes and EPAS1 genotypes (encoding hypoxia-inducible factor 2α) was only recently found. Also, scans for selection of candidate genes involved in the adaptation to high-altitude indicated that those genes were associated with hypoxia, energy metabolism, angiogenesis, Ca2 metabolism, cortisone synthesis, erythropoietin, and iron homeostasis (Wei et al., 2016). To our knowledge there are no data concerning acclimatization of shorn ewes to moderate altitude. Hypoxic exposure in rats is more pronounced in cold than in room temperature (Cadena and Tattersall, 2014). Lower critical temperature may change from 0 °C in a sheep with fleece to 20 °C for shorn sheep (Ames, 1980). At moderate altitude, in addition to hypoxia, there is also exposure to low environmental temperature. In this case, mammals attempt to maintain oxygenation and body temperature. These are potentially conflicting demands. Cold induces an increase in thermogenesis, and hypoxia suppresses metabolism conserving oxygen and preventing hypoxaemia (Cadena and Tattersall, 2014). Thus, increased maintenance requirements to maintain homeostasis could have negative effect on both animal health and production. In our earlier study we found hematocrit related differences in the pattern of hematological adjustments to moderate altitude in newly shorn ewes exposed to moderate altitude (1440 m above sea level) for four months (Moneva et al., 2016). However in that study hematological indices were measured only once (at d 14 following exposure to moderate altitude).

The object of the present study was to perform more detailed investigation on the physiological adjustments to moderate altitude in sheep having low, and high hematocrit values.

Materials and Methods

The present experiment involved Ile de France ewes that were selected by hematocrit values. The animals were allocated into two groups as follows: group I comprised animals with low level of hematocrit (n = 10) and group II comprised animals, which had high level of hematocrit (n = 10). The ewes were artificially inseminated in May following estrus synchronization. The average age of the ewes in group I and II was 4.9 ± 0.795 and 4.1 ± 0.745 years respectively.

The ewes were shorn in the end of May and were immediately transported from the experimental base of the Institute of Animal Science, Kostinbrod (500 m above sea level) to the Petrohan Pass region (Balkan Mountains), located at 1440 m above sea level. Minimum and maximum air temperatures on the day of arrival at the mountain pasture were 12.9 °C and 24.4 °C for the region of Kostinbrod (low altitude) and 8.0 °C–13,1 °C for the region of Petrohan Pass (moderate altitude) respectively. The animals stayed at high altitude for 4 months where they were on pasture for 10 h during the day. At night, they stayed in a barn. In addition to pasture, they were offered concentrate mixture once per day. The ewes had free access to a NaCl licking stone and water. Mean air temperature range in the region of Petrohan Pass during the summer months was 11.9 to 20.1 °C.

Blood samples were taken via jugular venipuncture within 3 min in the morning before feeding in order to minimize handling stress and avoid possible interference caused by cortisol diurnal variation. All samples were collected in EDTA tubes, centrifuged and stored at -20 °C until analyzed. Blood samples were collected at the following time points: before shearing, immediately after the transport, on day 5, 10, 30 and 60 after the transport. Hematocrit was measured by the microhematocrit method using EDTA-anticoagulated blood. Total erythrocyte count, and leukocyte count were determined by manual hemocytometer chamber count. Reticulocytes were stained with New methylene blue and counted microscopically. We followed the procedure described by Briggs and Bain (Dacie and Lewis, 2012). Three drops of the dye were delivered into a plastic tube by means of a plastic Pasteur pipette. The same volume of EDTA-anticoagulated blood was added to the dye solution and mixed. The mixture was kept at 37 °C for 20 min. Blood films were made on glass slides and were allowed to dry before being examined

without fixing. Plasma cortisol was measured using commercial cortisol ELISA kit 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). The results of one factor analysis are expressed as means ± S.E.M., and were analyzed by ANOVA.

Results and Discussion

Hematocrit levels declined significantly ($P < 0.001$) in both groups immediately after transportation of the sheep to the altitude pasture (Fig.1). Hematological adaptation of mountaineers to high altitude is associated with an initial peak of hemoglobin and hematocrit, attributed to increased diuresis that allows reduction in plasma volume and hemoconcentration caused by a shift of water out of the vascular system followed by a decrease during the first week (Mason, 2000; Tannheimer et al., 2009; Woods et al., 2011). The decline of hematocrit level after transport of the shorn sheep to the altitude pasture in our study was most probably due to severe cold experienced by the sheep because of the lack of wool insulation. In our earlier study, we found increased hematocrit level immediately after shearing at altitude of 500 m (Moneva et al., 2017). Similarly, shearing at altitude of 230 m and maximum ambient temperature of 45 °C has been reported to increase hematocrit level on the first day after the shearing (Casella et al., 2016). Also, increased hematocrit was reported in pigs exposed to heat (Waltz et al., 2014). Consequently, it could be argued that shearing-induced dehydration was followed by a transient hydration, which persisted after sheep unloading at the mountain pasture. Hematocrit levels increased significantly ($P < 0.01$) on d 5 in both groups as compared to baseline levels then declined slightly on d 10 and remained unchanged on d 30. Hematocrit levels increased significantly on d 60 in comparison with the values immediately after transport of the sheep to the mountain pasture, and were within the normal range (baseline levels) in the ewes of both groups. It is worth to note that the difference in hematocrit values between the groups persisted throughout

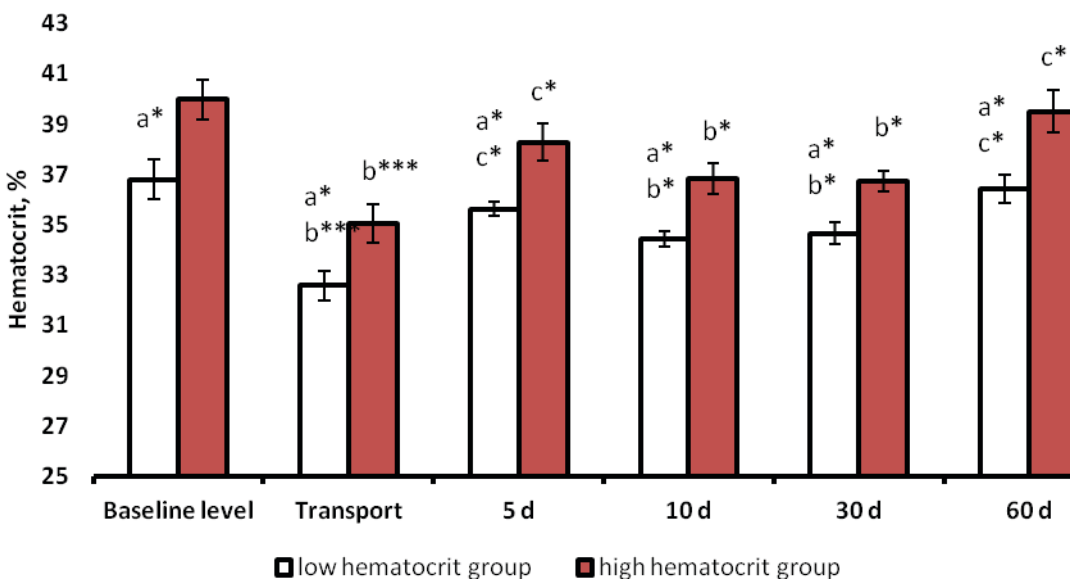

Fig. 1. Hematocrit in sheep with low and high baseline hematocrit values following exposure to moderate altitide * – $P < 0.05$; *** – $P < 0.001$;

a – significantly different among the groups;

b – significantly different versus baseline level;

c – significantly different versus transport to moderate altitude

the entire experimental period. Hematocrit levels suggest that the lack of wool insulation changed the expected increase in sheep hematocrit levels during the first 30 days on the mountain pasture. This view is consistent with the reported rate of wool growth (0.6 mm/day) during the summer months (Story and Ross, 1960) which suggest that at two months following shearing the expected wool length (3 см) would be enough to ensure satisfactory thermal insulation and reinstatement of hematocrit level.

Cortisol levels immediately after transportation of the ewes to the altitude pasture increased significantly in both groups. However, the increase of cortisol level was greater in the high hematocrit ewes (Fig. 2). Acclimatization to high altitude has been reported to cause initial increase of cortisol levels in high altitude visitors, but not in Sherpas (Park et al., 2014). Also, the authors found different cardiovascular responses to hypobaric hypoxemia between visitors to and natives of high altitude and suggested that hypothalamic-pituitary-adrenal axis and cardiovascular functions operate in synchrony under hypobaric hypoxemic conditions. This suggestion is consistent with the reported difference in cardiac innervation between lowlanders and Tibetans. Lowlanders exhibited sympathetic nervous system stimulation on acute exposure to hypoxia, whereas Tibetans expressed a significant vagal dominance (Zhuang et al., 1993; West et al., 2013). Consequently, the observed difference in cortisol level increment between the groups immediately after transport to altitude could be attributed to a possible difference in sympathetic nervous system stimulation since altitude increases sympathetic activation (Hainsworth et al., 2007) and acute hypoxia enhances muscle sympathetic activity (Duplain et al., 1999; Hansen and Sander, 2003). It was hypothesized that the high levels of NO exhibited by Tibetans in response to hypoxia served as a potent inhibitor of steroidogenesis on ascent to high altitude (Gilbert-Kawai et al., 2014). Therefore it could be speculated that NO was also involved in the lower adrenal response to altitude in low-hematocrit ewes. Plasma cortisol levels declined significantly on day 5 following exposure to altitude in both groups of sheep, then it tended to decline within the next 55 days and reached level

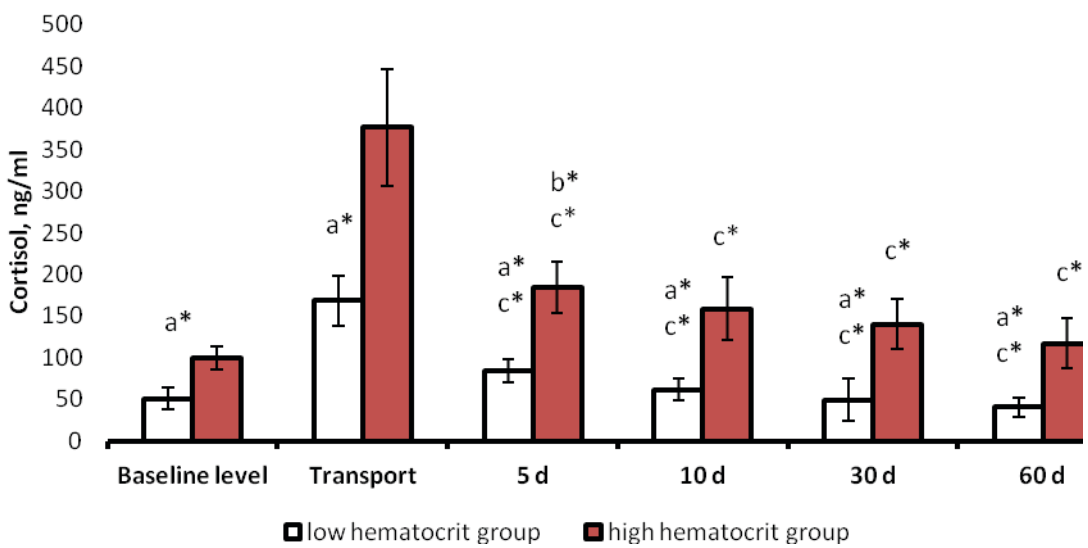

Fig. 2. Cortisol in sheep with low and high baseline hematocrit values following exposure to moderate altitide * – P < 0.05;

a – significantly different among the groups;

b – significantly different versus baseline level;

c – significantly different versus transport to moderate altitude;

d – significantly different versus 5 d after exposure to moderate altitude

of significance ($P < 0.05$) on d 60 relative to d 5 in low hematocrit group.

The observed difference in cortisol levels among the groups persisted throughout the entire experimental period. Thus, hematocrit-related difference in plasma cortisol dynamics between the groups may be associated to the specific metabolic changes found in high altitude natives along with the activity of autonomic nervous system divisions. Specifically, the Sherpas mitochondria are much more efficient at using oxygen and they demonstrate predilection toward carbohydrate oxidation with less reliance on lipid substrates which is related with an increase in the amount of ATP produced per volume of oxygen (Gilbert-Kawai et al., 2014). Furthermore, *m. vastus lateralis* in Tibetans unlike that in lowlanders exhibits increased prevalence of slow twitch fibers (Kayser et al., 1991; Kayser et al., 1996). Consequently, the low level of plasma cortisol in low hematocrit ewes could be attributed to diminished stimulation of sympathetic nervous system, prevalence of slow twitch muscle fibers and enhanced efficiency of respiratory chain that allowed them to maintain thermal homeostasis via production of more ATP per volume of oxygen. In contrast, high hematocrit ewes were expected to have less production of ATP per molecule of glucose and less effective oxygen utilization and therefore required increased synthesis of cortisol to stimulate gluconeogenesis and meet the increased demand of glucose under cold stress and hypoxia.

A general trend of higher leukocyte numbers in high hematocrit ewes relative to low hematocrit ewes was observed throughout the study (Fig. 3). This result is consistent with the current literature concerning the effects of catecholamines and cortisol on leukocyte distribution. It has been shown that catecholamines play a determining role during the first stage of the stress response and cause an increase of lymphocyte numbers within 30 min, whereas during the second phase, which occurs 2–4 hours later, dominate cortisol-induced neutrophil leukocytosis and lymphocytopenia (Shoenfeld et al., 1981; Benschop et al., 1996; Nakagawa et al., 1998; Olnes et al., 2016). There is enough data showing that the effect of catecholamines on lymphocytes is mediated via lymphocyte adrenoreceptors and depends on their density in the different leukocyte subpopulations (Benschop et al., 1996). Glucocorticoids have been shown to cause a shift of neutrophils from the marginated to the circulating pool with a minor contribution from marrow release (Nakagawa et al., 1998). Therefore the observed low cortisol level accompanied by a persisting trend of low white blood cell count in low-hematocrit ewes throughout the experimental period could be related with a possible difference in sympathetic activation between the animals of both groups, since Tibetans have been shown to exhibit less pronounced activation of sympathetic nervous system as compared to lowlanders (Gilbert-Kawai et al., 2014). However, the proposed interpretation does not explain leukocyte numbers increase ($P > 0.05$) observed on d 10 in the ewes of group I and on d 5 and 10 in the ewes of group II against the background of a significant cortisol decline in both groups as compared to the levels immediately after transport. This discrepancy between plasma cortisol level and leukocyte numbers could be due to the severe cold experienced by the animals during the first 10 d on the mountain pasture because of the lack of wool insulation.

Long-term exposure of mice on cold has been reported to cause peripheral leukocytosis due to persistent neutrophilia while heat exposure produced leukocytopenia (Novak et al., 1989). This view is consistent with the decline of leukocyte numbers on d 30 in both groups when wool length was expected to be approximately 18 mm and could provide a certain insulation against the cold. Leukocyte count in the ewes of group I on d 30 was significantly lower relative to that on d 10 while leukocyte count in the ewes of group II declined significantly on d 60, when wool length was expected to be 36 mm. Our results suggest that acclimatization to cold in the ewes of group I occurred 30 days earlier than in the ewes of group II. We can not exclude a possible effect of hypoxia on leukocyte numbers since intermittent hypoxia was found to cause short term increase in leukocyte numbers (Serebrovskaya et al., 2011). In our earlier paper (Moneva et al., 2019) we suggested a possible role of the tem-

perature-induced neutrophil elastase modulation which on its turn exerts control on corticosteroid binding globulin and ultimately regulates the level of free cortisol that is biologically active.

There was different pattern of reticulocytes dynamics between the groups. The number of reticulocytes in both groups of ewes increased at d 5 and then declined significantly at d 10 in high-hematocrit ewes while in low-hematocrit ewes reticulocyte numbers remained unchanged at d 10 relative to d 5. Besides, reticulocyte values in low-hematocrit ewes tended to be higher at d 30 as compared to high-hematocrit ewes (Fig. 4). It could be assumed that the expected increase

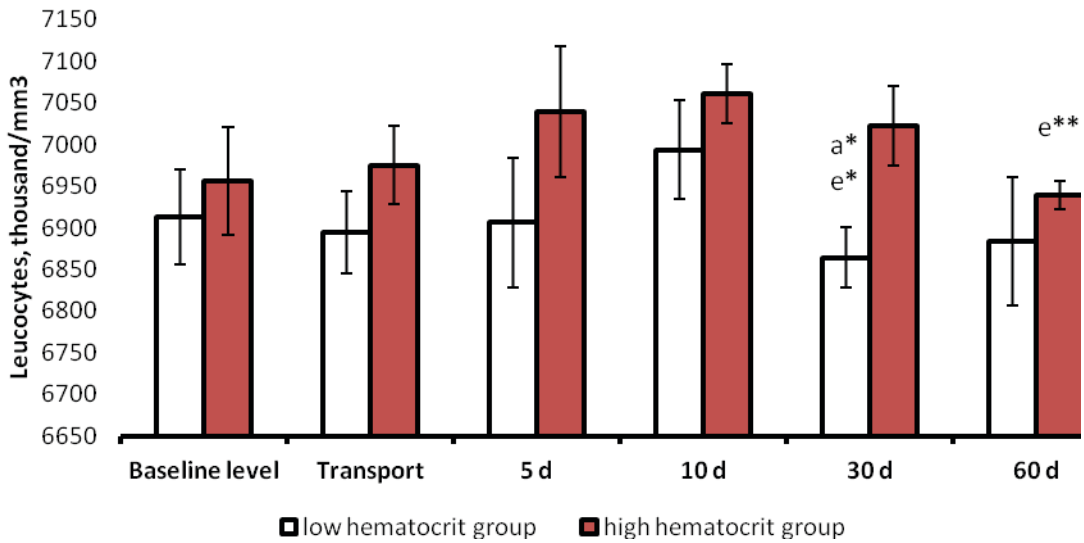

Fig. 3. Leukocytes in sheep with low and high hematocrit values following exposure to moderate altitude* – P < 0.05; ** – P < 0.01

a – significantly different among the groups;

e – significantly different versus 10 d after exposure to moderate altitude

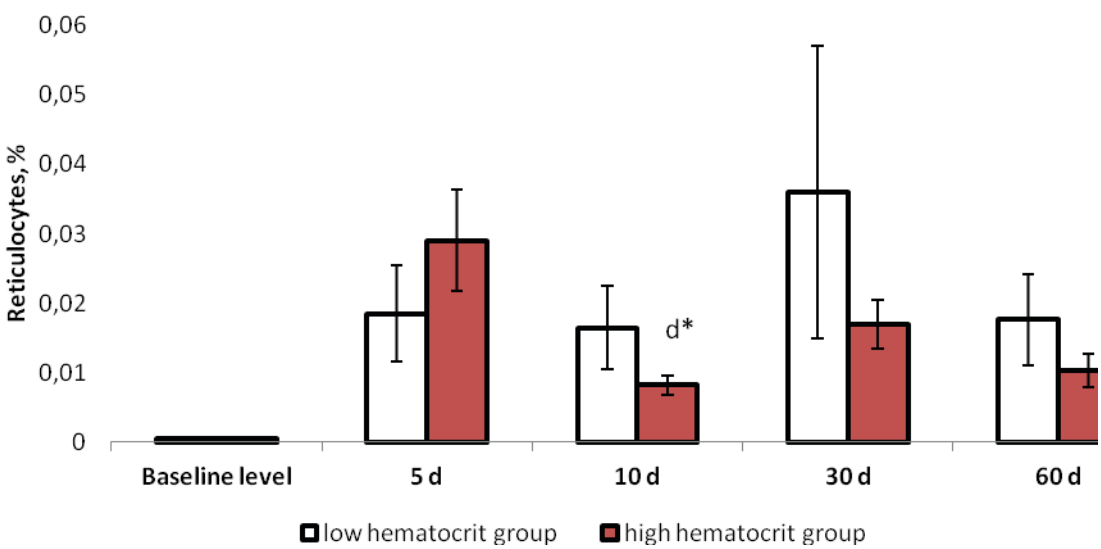

Fig. 4. Reticulocytes in sheep with low and high hematocrit values following exposure to moderate altitude.* – P < 0.05

d – significantly different versus 5 d after exposure to moderate altitude

in oxygen supply caused by the increase of reticulocyte numbers in low-hematocrit ewes at d 30 allowed them to increase their metabolic rate and consequently, to provide enough energy for maintenance of thermal homeostasis. This view is consistent with the observed significant decline of leukocyte numbers on d 30 in low-hematocrit ewes (Fig. 3), suggesting that the ewes didn't experienced cold stress at that time since cold is known to increase leukocyte numbers (Novak et al., 1989). In contrast, the unchanged number of reticulocytes in high-hematocrit ewes at d 30, accompanied with significantly higher leukocyte numbers as compared to low-hematocrit ewes, may signify that they experienced cold stress and therefore, the expected increased rate of cold air breathing could increase respiratory heat loss and deteriorate thermal homeostasis.

Erythrocyte count in low-hematocrit ewes tended to be low as compared to high-hematocrit ewes throughout the entire experimental period (Fig. 5). This is consistent with the effect of glucocorticoids on hematological changes under hypoxia. They stimulate erythropoiesis indirectly through upregulation of erythropoietin in the kidney (Fisher, 1998).

The observed trend in erythrocytes dynamics is in accordance with the established higher hematocrit values ($P < 0.01$) in the high-hematocrit ewes (Fig. 1). This discrepancy could be attributed to the observed trend of increased reticulocyte numbers at d 30 (Fig. 4) that was more obvious in low-hematocrit ewes. Reticulocytes are known to increase mean red cell volume since they are relatively large as compared to erythrocytes (Hill et al., 1987).

Erythrocyte numbers in both groups followed the same trend at d 60 as that observed in hematocrit dynamics.

The number of erythrocytes in both groups of ewes tended to decline at d 10 following exposure to altitude and reached significance at d 30 as compared to the values measured before transport (baseline), immediately after transport and at 5 d following exposure to altitude. The decline in the number of erythrocytes at d 30 is not consistent with the unchanged hematocrit values at d 10 and 30 (Fig. 1, 5).

Reticulocyte count dynamics along with the other hematological parameters indicated that the duration of hematological adjustments in newly

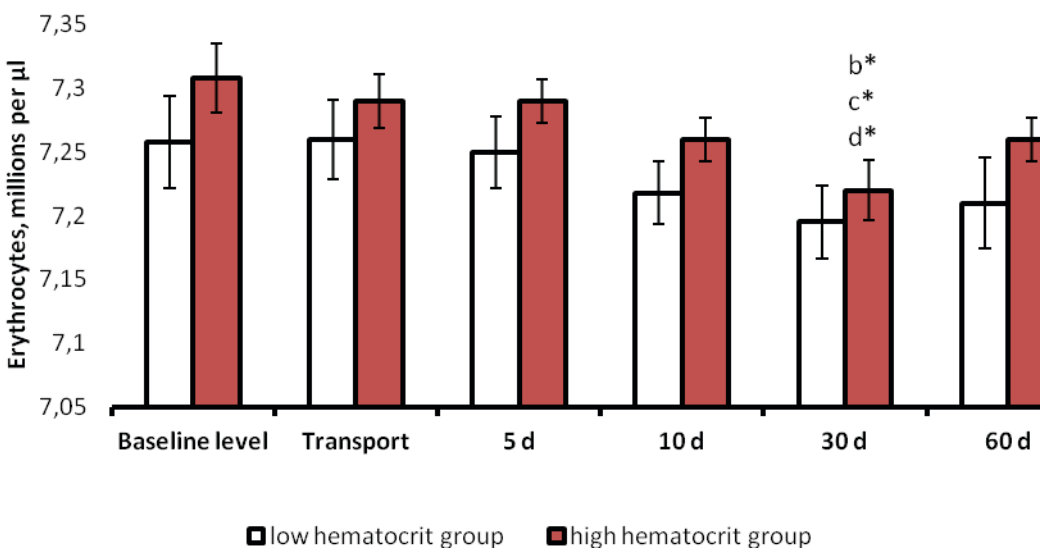

Fig. 5. Erythrocytes in sheep with low and high hematocrit values following exposure to moderate altitude * – $P < 0.05$

b – significantly different versus baseline level;

c – significantly different versus transport;

d – significantly different versus 5 day after exposure to moderate altitude

shorn ewes to altitude related hypoxia and cold stress was related to their hematocrit levels.

Conclusions

Exposure of newly shorn sheep to moderate altitude modified the expected hematological adjustments to moderate altitude hypoxia due to the severe cold experienced by the animals.

Sheep with low hematocrit level had different pattern of hematological adjustments to moderate altitude hypoxia accompanied by cold and showed earlier reinstatement of thermal homeostasis as compared to sheep with high hematocrit level.

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