Bulgarian Journal of Agricultural Science, 22 (No 6) 2016, 999–1005
Agricultural Academy

HEMATOCRIT AS A POTENTIAL MARKER OF ACCLIMATIZATION CAPACITY AND STRESS SENSITIVITY IN SHEEP EXPOSED TO TRANSPORT AND HIGH ALTITUDE

P. MONEVA*, I. YANCHEV, M. DYAVOLOVA and D. GUDEV
Agricultural Academy, Institute of Animal Science, BG - 2232 Kostinbrod, Bulgaria

Abstract

MONEVA, P., I. YANCHEV, M. DYAVOLOVA and D. GUDEV, 2016. Hematocrit as a potential marker of acclimatization capacity and stress sensitivity in sheep exposed to transport and high altitude. *Bulg. J. Agric. Sci.*, 22: 999–1005

The present study was conducted to examine the association between hematocrit, erythropoiesis and cortisol dynamics in the process of acclimatization to high altitude and during transport from high to low altitude. One hundred one Ile de France ewes were allocated into two groups following hematocrit measurement in all ewes. Group I comprised ewes with low level of hematocrit and group II comprised ewes with high level of hematocrit. Sheep were raised at low altitude (500 m above sea level). The ewes were shorn and immediately transported to high altitude (1440 m above sea level) where they were raised on pasture for 4 months during the grazing season. Blood samples were taken by jugular venipuncture several days before the shearing, at 14 d after exposure to high altitude, immediately after transport from high to low altitude and 7 days later. The following parameters were measured: hematocrit values, erythrocyte count, reticulocyte count and concentration of plasma cortisol. Hematocrit values remained unchanged in the ewes of group I through the analysis of all measurements whereas in the ewes of group II it tended to decline and reached level of significance at 7 d after transport to low altitude. Cortisol levels before shearing and by 14 d following exposure to high altitude were significantly higher in the ewes of group II as compared to the ewes of group I. Exposure to transport caused significant increase of plasma cortisol in the ewes of group I relative to baseline level whereas the increase of plasma cortisol in the ewes of group II was insignificant. Reticulocyte count at 14 d following exposure to high altitude was significantly higher in the ewes of group II as compared to the ewes of group I.

The results are interpreted to suggest that hematocrit is associated with the type of hemoglobin and predominant metabolic pathway for energy supply which ultimately predetermines the pattern of hematological changes during exposure to high altitude as well as sensitivity to transport stress.

Key words: cortisol, reticulocytes, high altitude, shearing, transport, stress, sheep

Introduction

It is well known that the main biological role of stress is to ensure a quick supply of glucose and allow the body to meet the increasing demand of energy during exposure to stress. A key adaptive response to chronic hypoxia is a switch from oxidative phosphorylation to anaerobic glycolysis (Gilany and Vafakhah, 2010). Exposure to high altitude increases blood lactate concentration compared with sea level values which results from greater reliance on anaerobic glycolysis (McArdle et al., 2010). Under conditions of hypoxia both hepatic lactate uptake and glucose production are increased (Palmer and Clegg, 2014). Therefore, an increase in Cori cycle activity under hypoxia can supply the necessary substrate to fuel the greater dependency of blood glucose brought about by increased hypoxia inducible factor (Palmer and Clegg, 2014). Increased gluconeogenesis under conditions of hypoxia serves to minimize accumula-

**Corresponding author*: pv_moneva@abv.bg

tion of lactate in muscles and provide adequate amounts of glucose to facilitate the switch in fuel utilization (Palmer and Clegg, 2014). Both acute and chronic exposure to high altitude activate sympathetic nervous system and hypothalamic-pituitary- adrenal axis (Bloom et al., 1976; Humpeler et al., 1980; Sawhney et al., 1991; Mazzeo et al., 1994, 1995, 2000). Glucocorticoids exert permissive effect on the stimulation of gluconeogenesis in the liver by glucagon and epinephrine (Exton, 1979). Also, glucocorticoids take part in the hematological changes under hypoxia. They stimulate erythropoiesis indirectly through upregulation of erythropoietin in the kidney (Fisher, 1998). Stress-induced erythropoiesis involves glucocorticoid regulation of erythroid progenitor expansion and terminal differentiation arrest (Bauer et al., 1999; von Lindern et al., 1999). Increased erythroid proliferation and differentiation arrest leads to increased production of immature red blood cells (Luger et al., 2003). At high altitude, in addition to hypoxia, there is also exposure to low environmental temperature. In this case, the respiratory system is influenced by conflicting factors because of the respiratory heat loss associated with an increase in ventilation during hypoxia (Diesel et al., 1990; Mortola and Frappell, 2000). Optimum hematocrit level for tissue oxygenation under hypoxic conditions is of great importance for animal adaptive capacity. Consequently, we aimed to investigate whether basal hematocrit level has any effect on adaptive capacity of sheep, following shearing and exposure to high altitude conditions.

Materials and Methods

One hundred one Ile de France ewes (1–7 years old) were used in the present experiment. All ewes of the flock were artificially inseminated in May following estrus synchronization. The animals were allocated into two groups following hematocrit measurement in all ewes. Group I comprised individuals with low level of hematocrit (low hematocrit group; n = 10) and group II comprised individuals which had high level of hematocrit (high hematocrit group; n = 10). Two additional measurement of baseline hematocrit at intervals of 10 days were performed in the sheep of both groups to verify hematocrit values of both groups, since hematocrit is known to be influenced by many factors and fluctuates from day to day. The average age of the ewes in groups I and II was 3.9± 0.795 and 3.1± 0.745 years respectively. The ewes were shorn on June 2nd 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 temperatures on that day were 13.9 and 25°C for the region of Kostinbrod (low altitude) and 8.2–13.6°C for the region of Petrohan Pass (high altitude) respectively. The animals remained at high altitude for 4 months where they were on pasture for 10 h during the day. At night they stayed in a barn. The ewes had free access to a NaCl licking stone and water. In addition to pasture, they were offered concentrate once per day. Mean air temperature range in the region of Petrohan pass during the summer months of 2015 was 12 to 20°C. At the end of the grazing season the ewes were transported back to low altitude. At that time the ewes in groups I and II were at 131±6.652 and 140±4.015 d of gestation respectively as estimated by the day of parturition.

All 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. Blood samples were collected in EDTA tubes, centrifuged and stored at –20°C until analyzed. Hematocrit was measured by the microhematocrit method using EDTA-anticoagulated blood. Total erythrocyte count was 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 15–20 min. Blood films were made on glass slides and were allowed to dry before being examined without fixing. Plasma cortisol was measured using a commercial cortisol ELISA kit. The results of one factor analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and Discussion

Plasma cortisol level in the ewes of group II (high baseline level of hematocrit) was significantly higher ($P < 0.05$) relative to that in group I (low baseline level of hematocrit) before transport to high altitude. The observed difference in the baseline cortisol levels could be due to difference in the hemoglobin types between the two groups. Hemoglobin has been found to determine or control the baseline levels of erythropoiesis, tissue 0_2 consumption, physical activity and behavior (Shirasawa et al., 2003). It has been speculated that individuals who carry variant hemoglobin with low 0_2 affinity can dissociate more 0_2 in the peripheral tissues whereas the other hemoglobin variant (having high 0_2 affinity) proceed with normal gas exchange in the lung (Shirasawa et al., 2003). Cortisol levels at 14 d following exposure to high al-

titude did not differ from the respective baseline levels at low altitude in both groups (Figure 1). Our experimental design did not include measurement of plasma cortisol during the first several days after arrival at high altitude. However, it is well documented that hypoxia of high altitude results in an increase in plasma cortisol and catecholamines with transient duration (Humpeler et al., 1980; Sawhney et al., 1991; Mazzeo et al., 1994).

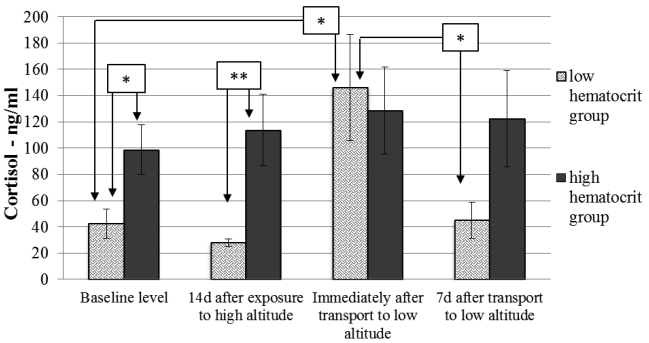

Fig. 1. Cortisol levels in sheep with low and high baseline hematocrit values following exposure to high altitude and transport to low altitude. * - p < 0.05; ** - p < 0.01

Exposure of calves to hypoxia has been reported to increase the output of cortisol and corticosterone to levels which occur in response to supramaximal doses of corticotrophin (Bloom et al., 1976). Physiological adaptation on immediate ascent to high altitude include hematologic changes and switch of the respiratory pathway to anaerobic glycolysis (Kambiz and Vafakhah, 2010; McArdle et al., 2010). The reported increase in plasma cortisol during the initial stage of acclimatization to high altitude is hardly coincidental. Increased gluconeogenesis under conditions of hypoxia serves to minimize accumulation of lactate in tissues and provide adequate amounts of glucose (Palmer and Clegg, 2014). Glucocorticoids are potent stimulators of gluconeogenesis and their increase during the first several days at high altitude are considered as an adaptive response to limited oxygen availability. Cortisol values at 14 d following exposure to high altitude were within the baseline range and suggest that the acclimatization period comprising the above mentioned physiological changes was less than 14 days. Presumably, increased gluconeogenesis during the first several days at high altitude requires high levels of cortisol to stimulate gluconeogenesis because of the greater reliance on gluconeogenesis during that period. It is unlikely to assume that the enumerated physiological changes immediately after ascent to altitude did not take place in our experiment because of the expected relatively mild hypoxia at an altitude of 1440 m. The main argument for this view is that altitude exposure in our experiment was accompanied with cold stress caused by sheep shearing immediately before transport to high altitude.

Physiological adjustments to hypoxia require increased ventilation and suppressed metabolism to provide oxygen whereas cold exposure requires an increase of thermogenesis and hypoventilation to reduce heat losses associated with breathing (Diesel et al., 1990; Cadena and Tattersall, 2014). These conflicting requirements exacerbate the features of hypoxia. Initial hypoxic exposure is more pronounced in cold than in room temperature (Cadena and Tattersall, 2014). The lower critical temperature (the ambient temperature below which the rate of heat production increases to maintain thermal balance) for adult sheep with 5 mm wool fed at maintenance diet is 25°C (Blaxter, 1967). Temperature range during the first day at high altitude in our study (8.2-13.6°C) was far below the lower critical temperature. Our view that the animals most probably experienced pronounced hypoxia during the first week after arrival at high altitude is further supported by reticulocytes dynamics. We did not find reticulocytes at low altitude in the animals of both groups. Reticulocyte count increased significantly after 14 d at high altitude (Figure 2). It is well documented that hypoxia-inducible factor and erythropoietin are major mediators in oxygen-regulated erythropoiesis (Haase, 2010).

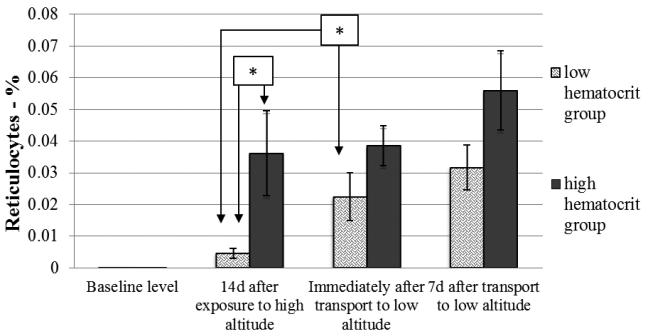

Fig. 2. Reticulocyte count in sheep with high and low baseline hematocrit values following exposure to high altitude and transport to low altitude. * - p < 0.05

There is evidence that glucocorticoids take part in erythropoiesis and have potential to stimulate proliferation of erythroid cell in the presence of limiting amounts of erythropoietin (Udupa et al., 1986). They stimulate erythropoiesis indirectly by increasing erythropoietin production in the kidney (Fisher, 1998). Besides, glucocorticoid receptor causes differentiation arrest of primary erythroid progenitors, thus increasing reticulocyte count (Wessely et al., 1997). This data is consistent with our view that cortisol, as a stimulator of

erythropoiesis, was most probably increased during the first week at high altitude due to the combined effect of transport, cold and hypoxia. Similar changes in reticulocytes have been reported in chickens selected for rapid growth coupled with low ambient temperature. The reported changes were attributed to retarded angiogenesis and insufficient oxygen supply (Luger et al., 2003). It is worth noting that reticulocyte count at 14 d after arrival at high altitude was significantly higher in the ewes with high hematocrit level as compared to that in the ewes with low hematocrit level (Figure 2). Elevated reticulocyte count is a reliable marker of increased erythropoiesis. The higher erythropoietic response in the second group suggests that each group employed different pattern of hematological adjustments to high altitude. The observed difference in reticulocyte response to high altitude could be attributed to difference in the hemoglobin types between the two groups. Earlier studies have shown that sheep with hemoglobin A have consistently higher hematocrit than sheep with hemoglobin B (Evans and Whitlock, 1964). Furthermore, mutant mice that carry hemoglobin variant with low oxygen affinity have been found to show increased oxygen consumption and $C0_2$ production in tissue metabolism, suggesting enhanced O_2 delivery (Shirasawa et al., 2013). Our assumption is further supported by the reported polycythemia in individuals who carry hemoglobins with high O_2 affinity (Jones et al., 1967; Grace et al., 1992). Therefore, it could be assumed that the animals in group II possessed hemoglobin with high O_2 affinity because of the higher baseline value of hematocrit and higher rate of erythropoiesis at 14 d following exposure to high altitude.

It is intriguing that hematocrit level at 14 d after arrival at high altitude was unchanged in the ewes of group I and tended to decline in the ewes of group II relative to their baseline levels despite the enhanced erythropoiesis as judged by the increased reticulocyte count at that time (Figure 3). It is necessary to trace specific hematogical adjustments back to the first day of exposure to high altitude in order to explain the observed discrepancy. The specific hematological changes immediately after arrival at high altitude were omitted in our study. However, they have been relatively well elucidated in the literature.

Hematological adaptation to high altitude is associated with an increase in hematocrit values that is attributed to a shift of water out of the vascular system (Mason, 2000; McArdle et al., 2010). The rapid reduction in plasma volume results in an increase in hemoglobin concentration that enables the body to compensate oxygen dependent energy deficit (Mason, 2000; Stark and Schuster, 2012). These hemodynamic adjustments are related to the activation of sympatico-adrenal system (Allen and Patterson, 1995; Parker et al., 2003). After a certain period of time, despite the continuing increase in erythropoiesis and red-cell mass, hemoglobin concentration starts to plateau off because of the increase in plasma volume (Mason, 2000). Consequently, taking into account the above mentioned, it can be assumed that the unchanged hematocrit level in the ewes of group I at 14 d following exposure to high altitude was due to reinstatement of plasma volume at that time following a supposed decrease in plasma volume immediately after arrival at high altitude.

Hematocrit reinstatement at 14 d could be considered as a marker of the beginning of a second stage in the acclimatization process, related with initial elevation of reticulocyte count in the first group of ewes. Most probably the second stage of acclimatization occurred earlier in the second group of ewes. Increased reticulocyte count during the second stage of acclimatization was expected to maximize oxygen delivery to the tissues due to the increased metabolic activity, higher 2,3-diphosphoglycerate, lower hemoglobin-oxygen affinity and higher deformability of the young red blood cells relative to senescent blood cells (Mairbäurl, 2013; Kaestner and Bogdanova, 2014).

The high reticulocyte count in the ewes of group II as compared to that in the ewes of group I at 14 d following exposure to high altitude may be associated with the type of hemoglobin. Our assumption that the ewes in group II carried hemoglobin with high 0_2 affinity suggests greater reliance on anaerobic glycolysis in comparison with the ewes of group I. Increased energy demand during hypoxia requires quick shift of the respiratory pathway to anaerobic glycolysis which is due to the fact that metabolism of glucose via glycolysis requires less oxygen as compared to oxidative phosphorylation (Palmer and Clegg, 2014). Although anaerobic glycolysis produces 18 times less ATP from each glucose molecule than does the aerobic system, the rate of ATP synthesis via glycolysis is around 100 times faster than oxidative phosphorylation. Increased reliance on glycolysis leads to accumulation of lactate and requires enhanced gluconeogenesis to minimize accumulation of lactate and provide adequate amounts of glucose. Consequently, the higher plasma concentration of cortisol in the ewes of group II at low and high altitude (Figure 1) is consistent with the assumption that the ewes with high baseline hematocrit values had higher reliance on glycolysis. On the contrary, the ewes of group I which were supposed to rely more on oxidative phosphorylation were expected to require less baseline levels of cortisol. Lower cortisol levels in this group before transport to high altitude and 14 d following exposure to high altitude are in agreement with this view.

The shift to anaerobic metabolism under stress conditions was expected to cause more pronounced stimulation

of glycolysis and gluconeogenesis in the ewes of group I because of their higher dependence on oxidative phosphorylation. Increased energy deficit in these ewes caused by transport stress would require fast production of energy via glucocorticoid stimulated glyconeogenesis. This assumption corresponds to the higher cortisol response found immediately after transport to low altitude as compared to baseline level (Figure 1). On the contrary, adrenal response to transport stress in the ewes of group II was insignificant. This was probably due to the preferential reliance on glycolysis and therefore to higher capacity for quick supply of energy in the ewes of group II. Besides, there was no significant difference in plasma cortisol levels between the groups immediately after transport to low altitude. Consequently, lower adrenal response to transport in the ewes of group II was due to their higher baseline level of cortisol, needed to support higher rate of glycolysis in the ewes of this group. Stress hyporesponsiveness during pregnancy is well documented in sheep (Young and Rose, 2002). Therefore, the observed difference in the adrenal responsiveness to transport can hardly be attributed to the difference in the gestational age among the groups at that time. Also, investigations concerning age related changes in adrenal responsiveness to stress have not been conclusive (Goncharova, 2013). Consequently, it is unlikely to assume that age differences whether within or between the groups might have influenced cortisol level immediately after transport to low altitude.

Cortisol levels in both groups returned to their baseline range by 7 d after transport to low altitude. The greater cortisol response to transport stress in the ewes of group I suggests that stress is closely related with the type of hemoglobin. Our view is consistent with the reported control of O_2 consumption, metabolism, and physical activity by oxygen affinity of hemoglobin (Shirasawa et al., 2003).

Hematocrit level has been reported to be associated with hypoxia-induced ascites in chickens, exposed to ascites -predisposing cold environment. High hematocrit in ascites chickens was reported to generate continuous increase of cortisol and erythropoiesis (Luger et al., 2003).

It is worth noting that hematocrit values and erythrocyte count in the ewes of group I remained unchanged at high altitude, following transport to low altitude and 7 days later whereas these blood parameters had opposite dynamics in the ewes of group II (Figures 3, 4). The observed changes in hematocrit and reticulocytes suggest that the higher rate of erythropoiesis in the ewes of group II required increased plasma volume as a means to diminish blood viscosity and improve oxygen delivery to the tissues. Another remarkable finding was that reticulocyte count in the ewes of group I immediately after transport to low altitude did not differ significantly from that of the ewes in group II (Figure 2). This was probably due to sluggish erythropoietic response in the ewes of group I over the 4 month long exposure to high altitude. This suggestion is based on the fact that it takes at least 15 hours after altitude ascent for erythropoietin to start erythropoiesis (McArdle et al., 2010) and around 2 days to reach peak response (Mason, 2000). Therefore, increased reticulocyte count in the ewes of group I immediately after transport was not caused by the transport itself.

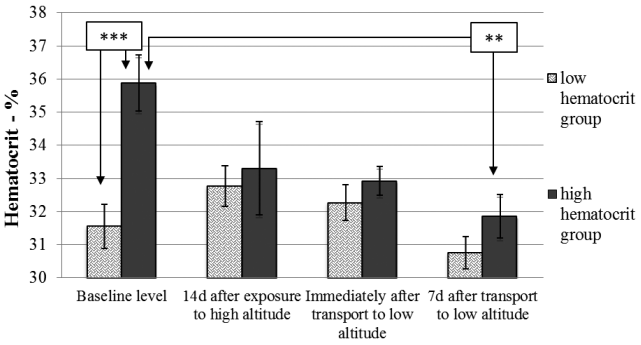

Fig. 3. Hematocrit values, following exposure to high altitude and transport to low altitude, in sheep with high and low baseline hematocrit values. ** - p < 0.01; * - p < 0.001**

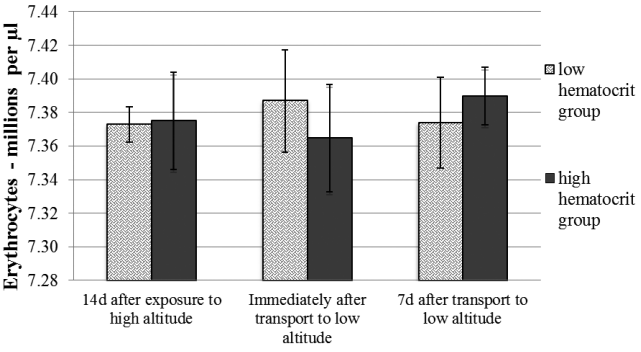

Fig. 4 Erythrocyte count in sheep with high and low baseline hematocrit values following exposure to high altitude and transport to low altitude

However, increased erythropoiesis in both groups during the exposure to high altitude did not correspond to the cortisol levels at that time which were similar to baseline levels. It is known that cortisol stimulates erythropoiesis and production of immature red blood cells that require higher cortisol level at that time (Wessely et al., 1997; Bauer et al., 1999). This discrepancy could be explained by the reported functional potentiation of glucocorticoid activity in hypoxia due to increased sensitivity to glucocorticoids (Leonard et al., 2005).

High hematocrit and hemoglobin levels have also been found in double-muscled cattle which suggest a possible association between skeletal muscle mass and hematocrit level (Fiems, 2012). Double muscling in cattle is accompanied by a marked change in the fiber composition from one reliant on mitochondrial oxidative metabolism to glycolysis (Puddick and Martinus, 2011). Myostatin-null mice (myostatin is a negative regulator of skeletal muscle mass) show a non-significant increase in serum levels of corticosterone with acute daily psychological stress as compared with wild type mice (Allen et al., 2010). They are predisposed to develop alveolar hypoxia and have lower fatigue resistance (Fiems, 2012).

Cortisol dynamics in the ewes of group II following transportation to low altitude (Figure 1) was similar to that reported in double- muscled animals (Allen et al., 2010) and supports the view that hematocrit was also associated with fiber- type predominance and the corresponding pathway for energy supply-oxidative metabolism or glycolysis. Unfortunately, our study did not include measurements of muscle growth and development. A double-muscling phenotype has been reported for animals of the Texel sheep breed (Cockett et al., 2005)

Our results suggest that hematocrit values in our study were associated with the pattern of adrenal and hematological changes in the process of acclimatization to high altitude and during transportation to low altitude. More investigations are needed to determine optimum hematocrit range that corresponds to increased capacity for acclimatization, adaptation, and stress resistance.

Conclusions

Ewes with high baseline values of hematocrit had higher plasma cortisol levels as compared to ewes with low baseline values of hematocrit. This association persisted at 14 d following exposure to high altitude.

Adrenal response to transport stress was more pronounced in ewes with low baseline values of hematocrit, whereas it was insignificant in ewes with high baseline hematocrit.

Reticulocyte count at 14 d following exposure to high altitude was significantly higher in ewes with high baseline values of hematocrit relative to ewes with low baseline hematocrit values.

References

Allen, D. L., G. E McCall, A. S Loh, M. C. Madden and R. S. Mehan, 2010. Acute daily psychological stress causes increased atrophic gene expression and myostatin-dependent muscle atrophy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **299**: R889-R898.

Allen, M. T. and S. M. Patterson, 1995 Hemoconcentration and stress: a review of physiological mechanisms and relevance for cardiovascular disease risk. *Biol Psychol.*, **41** (1): 1-27.

Bauer, A., F. Tronche, O. Wessely, C. Kellendonk, H. M. Reichardt, P. Steinlein, G. Schutz and H. Beug, 1999. *The glucocorticoid receptor is required for stress erythropoiesis. Genes Dev.*, **13***:* 2996-3002.

Blaxter, K. L., 1967. The Energy Metabolism in Ruminants**.** *Hutchinson and Son*, London.

Bloom, S. R., A. V. Edwards, R. N. Hardy and M. Silver, 1976. Adrenal and pancreatic endocrine responses to hypoxia in the conscious calf. *J Physiol.*, **261** (2): 271-283.

Cadena, V. and G. J. Tattersall, 2014. Body temperature regulation during acclimation to cold and hypoxia in rats. *J Therm Biol.*, **46**: 56-64.

Cockett, N. E., **M. A. Smit**, **C. A. Bidwell**, **K. Segers**, **T. L. Hadfield**, **G. D. Snowder**, **M. Georges and C**. **Charlier,** 2005. The callipyge mutation and other genes that affect muscle hypertrophy in sheep. *Genet Sel Evol.*, **37** (Suppl 1): S65-81.

Dacie, J. V. and S. M. Lewis, 2012. Practical Haematology, chapter 3, Churchil Livingstone (Ed.), *Elsevier*, p. 33.

Diesel, D. A., A. Tucker and D. Robertshaw, 1990. Cold-induced changes in breathing pattern as a strategy to reduce respiratory heat loss. *J Appl. Physiol.***, 69** (6): 1946-1952.

Evans, J. V. and J. H. Whitlock, 1964. Genetic Relationship between Maximum Hematocrit Values and Hemoglobin Type in Sheep. *Science*, **145** (3638): 1318.

Exton, J. H., 1979. Regulation of gluconeogenesis by glucocorticoids. *Monogr. Endocrinol.*, **12**: 535-546.

Fiems, L. O., 2012. Double muscling in cattle: genes, husbandry, carcasses and meat. *Animals*, **2** (3): 472-506.

Fisher, J. W*.,* 1998. *A quest for erythropoietin over nine decades. Annu. Rev. Pharmacol. Toxicol*., **38***:* 1-20.

Gilany, K. and M. Vafakhah, 2010. Hypoxia: a Review. *Journal of Paramedical Sciences* (JPS),.**1** (2): 43-60.

Goncharova, N. D., 2013. Stress responsiveness of the hypothalamic-pituitary-adrenal axis: age-related features of the vasopressinergic regulation. *Front Endocrinol (Lausanne)*, **12** (4): 1-15.

Grace, R. J., P. A. Gover, D. F. Treacher, S. E. Heard and T. C. Pearson, 1992. Venesection in haemoglobin Yakima, a high oxygen affinity haemoglobin. *Clin Lab Haematol.*, **14** (3): 195-199.

Haase, V. H., 2010. Hypoxic regulation of erythropoiesis and iron metabolism. *Am. J. Physiol Renal Physiol*., **299** (1): F1-13.

Humpeler, E., F. Scrabal and G. Bartsch, 1980. Influence of exposure to moderate altitude on the plasma concentration of cortisol, aldosterone, renin testosterone and gonadotropins. *Eur. J. Appl. Physiol.*, **17**: 167-176.

Jones, R. T., E. E. Osgood, B. Brimhall and R. D. Koler, 1967. Hemoglobin Yakina. I. Clinical and biochemical studies. *J Clin. Invest.*, **46** (11): 1840-1847.

Kaestner, L. and A. Bogdanova, 2014. Regulation of red cell lifespan, erythropoiesis, senescence, and clearance. *Front Physiol*., **5**: 269.

Leonard, M. O., C. Godson, H. R. Brady and C. T. Taylor, 2005. Potentiation of glucocorticoid activity in hypoxia through induction of the glucocorticoid receptor. *J. Immunol*., **174** (4): 2250-2257.

Luger, D., D. Shinder, D. Wolfenson and S. Yahav, 2003. Erythropoiesis regulation during the development of ascites syndrome in broiler chickens: A possible role of corticosterone. *J. Anim. Sci.*, **81**: 784-790.

Mairbäurl, H., 2013. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. *Front Physiol.*, **4**: 332- 345.

Mason, N. P., 2000. The physiology of high altitude: an introduction to the cardio-respiratory changes occurring on ascent to altitude. *Current Anaesthesia & Critical Care,* **11** (1): 34-41.

Mazzeo, R. S., G. A. Brooks, G. E. Butterfield, D. A. Podolin, E. E. Wolfel and J. T. Reeves, 1995. Acclimatization to high altitude increases muscle sympathetic activity both at rest and during exercise. *Am J. Physiol.*, **269**: R201-207.

Mazzeo, R. S., A. Child and G. E. Butterfield, 2000. Sympathoadrenal responses to submaximal exercise in women after acclimatization to 4000 m. *Metabolism*, **49**: 1036-1042.

Mazzeo, R. S., E. E. Wolfel, G. E. Butterfield and J. T. Reeves, 1994. Sympathetic responses during 21 days at high altitude (4300 m) as determined by urinary and arterial catecholamines. *Metabolism*, **43**: 1226-1232.

McArdle, W. D., F. I. Katch and V. L. Katch, 2010. Exercise at medium and high altitude, In: Exercise Physiology: Nutrition, Energy, and Human Performance, *Lippincott Williams & Wilkins*, chapter **24:** 592-608.

Mortola, J. P. and P. B. Frappell, 2000. Ventilatory responses to changes in temperature in mammals and other vertebrates. *Annu. Rev. Physiol.,* **62**: 847-874.

Palmer, **B. F. and D. J. Clegg**, 2014. Ascent to altitude as a weight loss method: the good and bad of hypoxia inducible factor activation. *Obesity (Silver Spring)*, **22** (2): 311-317.

Parker, A. J., G. P. Hamlin, C. J. Coleman and L. A. Fitzpatrick., 2003. Dehydration in stressed ruminants may be the result of a cortisol-induced diuresis. *J Anim. Sci.*, **81** (2): 512-519.

Puddick, J. and R. D. Martinus, 2011. Comparative proteomics of skeletal muscle mitochondria from myostatin-null mice. *Cell Biol. Int. Rep.*, **18**: 35-41.

Sawhney, R. C., A. S. Malhotra and T. Singh, 1991. Glucoregulatory hormones in man at high altitude. *Eur. J. Appl. Physiol.*, **62**: 286-291.

Shirasawa, T., M. Izumizaki, Y. Suzuki, A. Ishihara,T. Shimizu, M. Tamaki, F. Huang, K. Koizumi, M. Iwase, H. Sakai, E. Tsuchida, K. Ueshima, H. Inoue, H. Koseki, T. Senda, T. Kuriyama, and I. Homma, 2003. Oxygen affinity of hemoglobin regulates O_2 consumption, metabolism, and physical activity. *The Journal of Biological Chemistry*, **278** (7): 5035-5043.

Stark, H. and S. Schuster, 2012. Comparison of various approaches to calculating the optimal hematocrit in vertebrates. *J. Appl. Physiol***., 113** (3): 355-367.

Udupa, K. B., H. M. Crabtree and D. A. Lipschitz, 1986. In vitro culture of proerythroblasts: characterization of proliferative response to erythropoietin and steroids. *Br. J. Haematol*., **62** (4): 705-714.

von Lindern, M., W. Zauner, G. Mellitzer, P. Steinlein, G. Fritsch, K. Huber, B. Lowenberg and H. Beug, 1999. The glucocorticoid receptor cooperates with the erythropoietin receptor and c-Kit to enhance and sustain proliferation of erythroid progenitors in vitro. *Blood*, **94**: 550-559.

Wessely, O. E.-M. Deiner, H. Beug and M. von Lindern, 1997. The glucocorticoid receptor is a key regulator of the decision between cell-renewal and differentiation in erythroid progenitors. *The EMBO Journal*, **16** (2): 267-280.

Young, S. F. and J. C. Rose, 2002. Attenuation of corticotropin-releasing hormone and arginine vasopressin responsiveness during late-gestation pregnancy in sheep. *Biol. Reprod*., **66** (6): 1805-1812.

Received February, 29, 2016; accepted for printing November, 1, 2016