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Effect of altitude on some hematological parameters in two Bulgarian local sheep breeds depending also on their body condition score

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Abstract

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The aim of the present study was to estimate the values of some blood parameters – red blood cells (RBC) count, hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean erythrocyte volume (MCV) of two herds of Bulgarian local sheep breeds (closely related genetically), reared at different altitudes in one region, depending on their body condition score (BSC), too. The research was carried out in a farm in Mirkovo village (715 m above sea level) with *Sofia (Elin Pelin) local sheep* breed and in a farm in Gubesh village (1040 m above sea level) *with Central Stara Planina (Balkan) sheep* breed.

From these two herds of local sheep (1.5-7.5 years old) were selected age-matched groups consisted by 50 animals each, from which blood samples were taken. The body condition score was performed during sampling by one individual using an adapted system. For the purposes of the research, like in our previous researches and because of narrow individual variation, formed subgroups of 10 animals each with the highest and lowest HCT in the analysis of the samples, were also compared.

The BCS of sheep from the herd of *Central Balkan sheep* breed (Gubesh village) was better than that those from *Elin Pelin* breed (Mirkovo village), as the difference was significant between the herds in the three groups – overall for the herds ($P < 0.001$) and in the groups with high ($P < 0.001$) and low ($P < 0.01$) HCT. The higher altitude in the village of Gubesh together with the better BCS in this herd significantly influenced the levels of HCT in the three groups again ($P < 0.001$, $P < 0.001$, and $P < 0.01$) and MCV – $P < 0.05$, $P < 0.001$, and $P < 0.05$, respectively.

The levels of HGB were significantly higher overall for the herds ($P < 0.05$) and in the group with high HCT ($P < 0.05$) but the differences in RBC and MCH between all groups were not significant.

About the MCHC – there was a high significant difference ($P < 0.001$) between the breeds in all three groups – overall for the herds and in the groups with high and low HCT which indicates about a specific adaptive response of the sheep's organism from *Elin Pelin sheep* breed to maintain a higher concentration of HGB with a smaller volume of erythrocytes for almost the same number of cells.

Keywords: Local sheep; altitude; BCS, hematocrit; hematological parameters

Introduction

The diverse ecological and economic conditions in Bulgaria and the various needs and interests of the local people, allowed raising a large number of sheep breeds in the past. These local breeds are good adapted to the local conditions of climate and relief for the different areas. Although they all share a common origin, their environment and cultivation technologies, as well as specialization in different productive areas, have led to some genetic distance. However, with

a view to preserving these breeds, nowadays their range of distribution within Bulgaria is relatively wide, and in parallel with global climate changes, they inevitably undergo various acclimatization changes. Hence the need for new research in these breeds to take into account the adaptive reactions of the local sheep breeds has appeared. Such modern research is done by Mihailova et al. (2023) who investigated the genetic diversity and population structure of 50 flocks of 12 indigenous sheep breeds, including the two native breeds subject to the present study and previously unstudied. The low genetic differentiation between these breeds found by the authors, in parallel with their recommendations for improving the selection policy, is a reason for deepening the studies in other directions as well, such as hematological and biochemical studies of the herds, which also take into account the environmental factors.

Hemoglobin has been found to determine or control baseline levels of erythropoiesis, tissue oxygen consumption, physical activity and behavior (Shirasawa et al., 2003). It has been suggested that sheep carrying a hemoglobin variant with a low oxygen affinity can dissociate more oxygen in peripheral tissues, while the other hemoglobin variant (with a high oxygen affinity) continues with normal gas exchange in the lung (Shirasawa et al., 2003). Earlier studies showed that hemoglobin A sheep had a consistently higher hematocrit than hemoglobin B sheep and individuals keep HCT levels in narrow individual variations (Evans and Whitlock, 1964).

During the breeding of these local sheep over the years in our country, the so-called „transhuman pastoralism" – a traditional sheep management strategy was adopted by shepherds to cope with environmental stress at different altitudes during the spring-summer and autumn-winter breeding periods. A typical example in this regard is the study by Barsila et al, (2020) on local *Baruwal* sheep in the Himalayas, where blood samples were taken from specific groups at both grazing sites, from low (2431 m a.s.l.) to high grassland (3885 m a.s.l.), respectively and the results showed that RBC, HGB and PCV were significantly increased at high altitude ($p < 0.05$), while MCH and MCHC were higher at low altitude. In our similar previous study (Moneva et al., 2016), the relationship between hematocrit, erythropoiesis and cortisol dynamics in the process of acclimation to high altitude and during transport from high to low altitude in *Ile de France* ewes has also been investigated. The results therein are interpreted to suggest that hematocrit is related to hemoglobin type and the predominant metabolic pathway for energy supply, which ultimately predetermines the pattern of hematological changes during exposure to high altitude, as well as the sensitivity to transport stress.

A lot of researchers have indicated that the normal physiological values of various hematological parameters in farm animals are influenced by many factors such as age, sex, breed, season, altitude, climate, feeding and rearing conditions and many other environmental factors (Šoch et al., 2011, Macrae, 2017, Rahman et al., 2018). Hematological parameters on the other hand are useful tools to detect some changes in the health and physiological state of the animal that cannot be detected by physical examination (Badawi and Al-Hadithy, 2014). Determining the animal's current health status may have low reliability without hematological parameters, as these parameters allow for a more accurate diagnosis. Ahmadi-hamedani et al. (2016) reported that the determination of hematological reference values is a very important step to form a basis for clinical interpretation. Moreover, many researchers found, for example, that the level of RBC, PCV and HGB in diseased animals were significantly lower than healthy animals (Al-Samarai and Al-Jbory, 2017).

It is widely accepted that the Body Condition Score (BCS) of sheep has many advantages over live weight for determining animal condition and welfare (Kenyon et al., 2014). In our previous study (Metodiev et al., 2021), we attempted to evaluate the values of some blood parameters of *Ile de France* sheep according to their body condition score. BCS was found to significantly affect RBC ($P < 0.01$), HCT ($P < 0.05$) and HGB ($P < 0.05$). The data suggest that hematocrit levels are an important determinant of achieving a more favorable proportion of animals with optimal versus low BCS in the herd, especially in the presence of adverse factors such as parasite infestation and suspected malnutrition.

A crucial priority is to define the standard parameters for non-stressed animals reared in each individual country and/or geographic and climatic zone, by building a reference table with local data of hematological parameters, which should be updated periodically to make a proper comparison (Bezerra et al., 2010, Greguła-Kania et al., 2021). On the other hand, the study of literary sources related to the influence of extreme values of environmental factors on hematological and biochemical indicators can provide valuable information about the adaptation possibilities of selected breed. A similar profile of the *Avasi* breed in Jordan was made by Jawasreh et al. (2009) who fixed the variation in blood parameters in the studied animals due to several factors such as altitude, management, feeding level, age, sex, breed, health status, sample collection method, hematological techniques used, diurnal and seasonal variations of environmental temperature and physiological status of the animal as the objectives of this study were to evaluate the effect of intensive selection

and genetic manipulation on normal hematological and biochemical values in healthy adult, non-pregnant ewes.

In the context of the studies cited above, the aim of the present study was to estimate the values of some blood parameters – - red blood cells (RBC) count, hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean erythrocyte volume (MCV) of two herds of Bulgarian local sheep breeds (closely related genetically), reared at different altitudes in one region, depending on their body condition score (BSC), too.

Material and Methods

The research was carried out in a sheep farm in Mirkovo village (715 m above sea level) with *Sofia (Elin Pelin) local sheep* breed and a sheep farm in the village of in Gubesh village (1040 m above sea level) with *Central Stara Planina (Balkan) sheep* breed, in June 2022. Both herds of local sheep (1.5-7.5 years old) were reared traditionally for the breed on pasture during the sampling period, with additional feeding with concentrated feed (barley) 300 g per day, the ewes were milked twice a day. The shearing campaigns were not carried out before sampling, yet. From all sheep in the respective herds, were selected age-matched groups consisted by 50 animals each, from which blood samples were taken by jugular venipuncture in tubes with anticoagulant EDTA, which were transported in a thermally insulated container at a temperature of +4 °C. The body condition score was performed at the time of sampling by one individual according to the Jeffreies system adapted from Todorov et al. (1994).

Blood analysis was performed with a 5-Part-Diff Automated Hematology Analyzer (laser multidimensional hematology analyzer) URIT-5160, which allows simultaneous determination of 34 hematological parameters: WBC, LYM%, MON%, NEU%, EOS%, BAS%, LYM#, MON#, NEU#, EOS#, BAS#,RBC, HGB, HCT, MCV, MCH, MCHC, RDW_ CV, RDW_ SD, PLT, MPV, PDW, PCT,P_ LCR, P_ _LCC, RETIC%, RETIC_ _ABS, IRF. The methods by which the analyzer works in relation to the parameters included in the present study are: RBC/PLT analysis – by impedance method and HGB test – colorimetry with a reagent without cyanides.

Results are expressed as mean values ± S.E.M. and were analyzed statistically by the analysis of variance (ANOVA) method. For the purposes of the research, like in our previous researches and because of genetically determined narrow individual HCT variations, formed subgroups of 10 animals each with the highest and lowest HCT in the analysis of the samples, were also compared.

Results and Discussion

The Body Condition Score of the *Central Balkan sheep* from the herd of Gubesh village is better than that of Elin Pelin sheep from the village of Mirkovo **(Fig. 1)** – there are significant differences between the three groups – overall for the herds ($P < 0.01$) and in the high ($P < 0.001$) and low hematocrit groups ($P < 0.01$). The optimal BCS before insemination should be 3.0-3.5, because ewes with a relatively higher BCS, in the transition from seasonal anestrus to the breeding season, show significantly higher sexual activity than other ewes (Forcada and Abecia, 2006). Other authors (Khalil et al., 2022) indicated in their study that the physiological, biochemical, hematological status and productivity of *Naeemi* sheep in Kuwait were influenced not only by the seasons but also by their body condition score.

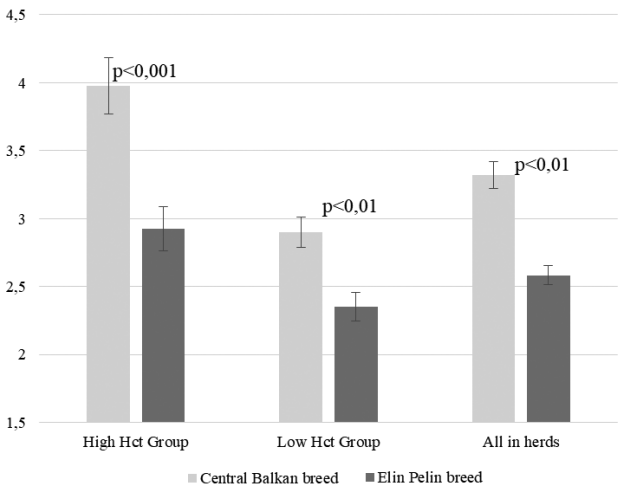

Fig. 1. BCS in two Bulgarian local sheep breeds (Range 1-5)

The HCT levels of the *Central Balkan sheep* were also higher than those of *Elin Pelin sheep* and differences between the three groups – overall for the herds ($P < 0.001$) and in the high ($P < 0.001$) and low HCT groups ($P < 0.01$) were significant again **(Fig. 2)**. This is in assonance to the expected higher levels of HCT in the Central Balkan sheep from the village of Gubesh, which are reared on a higher altitude, because there, in addition to hypoxia, there is also exposure to a lower temperature of the environment. In this case, the respiratory system could be affected by other environmental factors due to respiratory heat loss associated with increased ventilation during hypoxia (Mortola and Frappell, 2000). Hematological adaptation to high altitude is associated with an increase in HCT values, which is due to the displacement

of water from the vascular system (Mason, 2000; McArdle et al., 2010). The rapid decrease in plasma erythrocyte volume leads to an increase in hemoglobin concentration, which allows the body to compensate for the oxygen-dependent energy deficit (Mason, 2000; Stark and Schuster, 2012). After a certain period of time, despite the continued increase in erythropoiesis and the mass of red blood cells, the concentration of HGB begins to remain at a kind of plateau due to the increase in plasma volume (Mason, 2000). Therefore, taking into account the above, we can assume that in our case it is not a matter of short-term adaptation after transportation to a higher altitude, but of acclimatization to it that has already taken place. An additional possible logical explanation can be found in the above results for the BCS, which suggest a stronger metabolism in the *Central Balkan sheep* from the village of Gubesh for its maintenance, respectively, the need for more oxygen for metabolic processes.

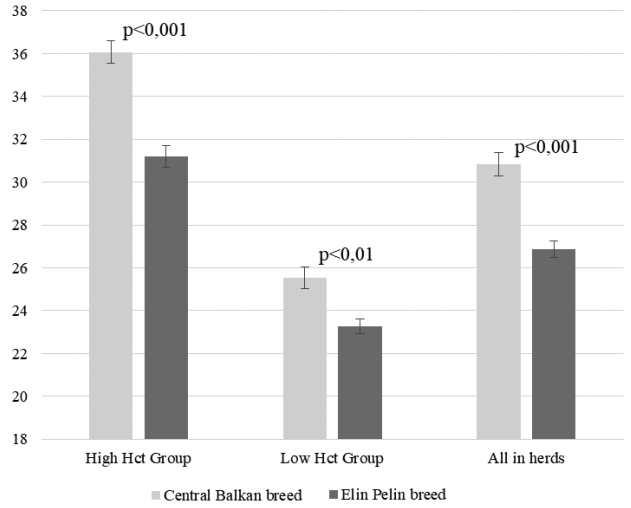

Fig. 2. Hematocrit levels in two Bulgarian local sheep breeds (%)

Absolutely the same differences and trends are logically observed in terms of HGB and RBC count with one exception **(Fig. 3 and Fig. 4)**. There was a significantly higher hemoglobin level in the high HCT sheep groups and totally the herds ($P < 0.05$). However, a certain impression is made by the almost equal values of the number of erythrocytes in the low HCT group, as well as the lack of significant differences in the remaining two groups. This is a direct indication of an increased concentration of hemoglobin in the erythrocytes, and we think it is because it also supports our above hypothesis of an increased metabolism. There is evidence that glucocorticoids are involved in erythropoiesis and have the potential to stimulate erythroid cell proliferation in the presence of limited amounts of erythropoietin. They stimulate erythropoiesis indirectly by increasing the production of erythropoietin in the kidneys. Increased erythroid proliferation and differentiation arrest leads to increased production of immature red blood cells (Luger et al., 2003).

There were significant differences between the mean values of the two herds ($P < 0.05$), as well as those of the low ($P < 0.05$) and high ($P < 0.001$) HCT groups for the

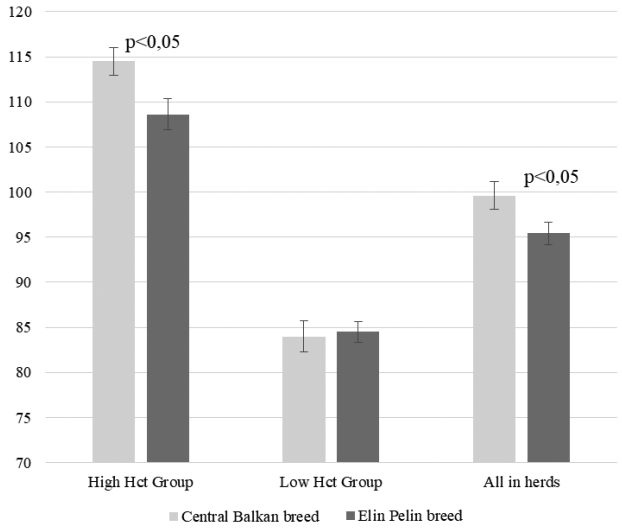

Fig. 3. Hemoglobin leves in two Bulgarian local sheep breeds (g/L)

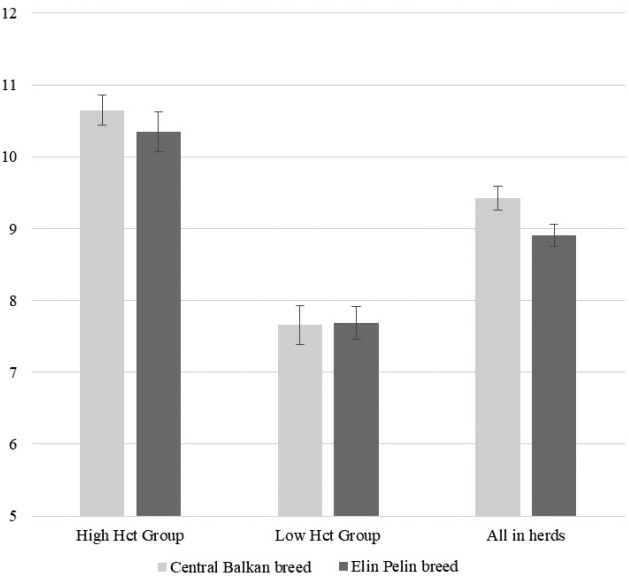

Fig. 4. RBC number in two Bulgarian local sheep breeds (10^12/L)

mean corpuscular volume of erythrocytes (MCV). We observed here the same trend as about the data for HCT and HGB, with higher MCV values being registered in the *Central Balkan sheep* from the village of Gubesh again **(Fig. 5)**. However, for the mean corpuscular hemoglobin (MCH) parameter, there were no significant differences between both sheep breeds in the three groups **(Fig. 6)**, as far as different results were obtained for them about the higher mean values in addition to high deviations. In the context of the discussion above regarding hematocrit, we again come to the conclusion reported by Mason (2000) for the retention of hemoglobin concentration with increased erythrocyte volume as an indication of a completed acclimation process to the altitude as an environmental factor. It should be noted that all average levels of these indicators, including deviations, are closely around the average reference values for sheep, within the limits reported in the book by Radostits et al. (2000).

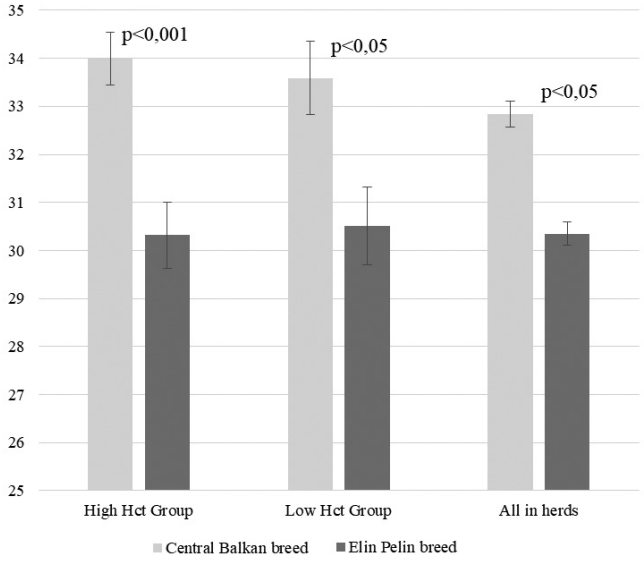

Fig. 5. MCV levels in two Bulgarian local sheep breeds (fL)

The trends in all the parameters discussed so far definitely result in the mean corpuscular hemoglobin concentration (MCHC) parameter, where there is a high significant difference ($P < 0.001$) between the three groups – overall for the herds and in the high and low hematocrit groups but this time the significantly higher concentration of hemoglobin in erythrocytes we found in the *Elin Pelin sheep* breed **(Fig. 7)**. The mean corpuscular hemoglobin concentration (MCHC) is an indicator of the concentration of hemoglobin in red blood cells. Because hemoglobin is the molecule to which oxygen binds, MCHC is a measure of the average oxygen-carrying capacity of the red blood cells circulating in the body. Low MCHC values (hypochromia) may mean that there is a lower concentration of hemoglobin within a given volume of red blood cells and, therefore, a reduced capacity to carry oxygen to the body's tissues. High MCHC values (hyperchromia) may mean that there is a higher than normal con-

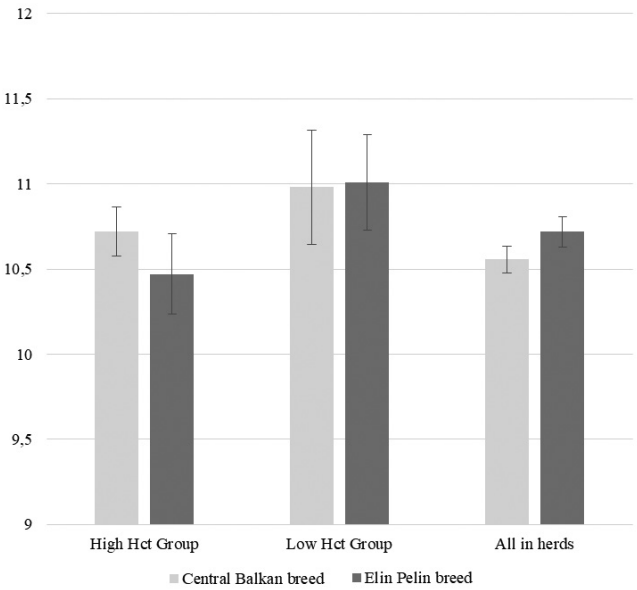

Fig. 6. MCH levels in two Bulgarian local sheep breeds (pg)

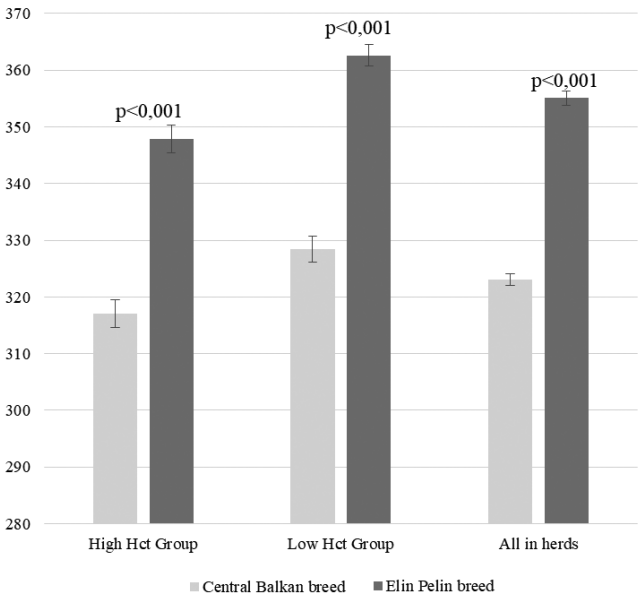

Fig. 7. MCHC levels in two Bulgarian local sheep breeds (g/L)

centration of hemoglobin in the red blood cells. The MCHC value is useful in the diagnosis of anemia, but must be used and interpreted in conjunction with the red blood cell count and other values in the complete blood count (CBC) related to red blood cells, such as the mean erythrocyte volume (MCV) and red blood cell distribution width (RDW), for the correct diagnosis of various health problems. All average levels of these indicators, including the deviations are also closely around the mean reference values for sheep, according to Radostits et al. (2000). In this context, in accordance with the rest of the hematological parameters studied above, the levels of MCHC we found may be an indication of a specific adaptive response of the sheep's organism from *Elin Pelin sheep* breed to maintain a higher concentration of hemoglobin with a smaller volume of erythrocytes for almost the same number of cells.

In conclusion, we can note that the hematological indicators we studied after differentiation and of subgroups of sheep in terms of hematocrit levels can give valuable scientific information about the reaction of animals to the "altitude" factor at different BCS of the studied herds from these two local sheep breed (closely related genetically), taking into account the influence of other relevant stress factors for the season such as shearing campaign and temperature differences.

Conclusions

The BCS of sheep from the herd of *Central Balkan sheep* breed (Gubesh village) was better than that those from *Elin Pelin* breed (Mirkovo village), as the difference was significant between the herds in the three groups – overall for the herds ($P < 0.001$) and in the groups with high ($P < 0.001$) and low ($P < 0.01$) HCT.

The higher altitude in the village of Gubesh together with the better BCS in this herd significantly influenced the levels of HCT in the three groups again ($P < 0.001$, $P < 0.001$, and $P < 0.01$) and MCV – $P < 0.05$, $P < 0.001$, and $P < 0.05$, respectively.

The levels of HGB were significantly higher overall for the herds ($P < 0.05$) and in the group with high HCT ($P < 0.05$) but the differences in RBC and MCH between all groups were not significant.

About the MCHC – there was a high significant difference ($P < 0.001$) between the breeds in all three groups – overall for the herds and in the groups with high and low HCT which indicates about a specific adaptive response of the sheep's organism from *Elin Pelin sheep* breed to maintain a higher concentration of HGB with a smaller volume of erythrocytes for almost the same number of cells.

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