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Differences in some biochemical parameters in two herds of Karakachan sheep depending on environment

Ivan Yanchev*, Nikola Metodiev, Penka Moneva and Maya Ignatova
Agricultural Academy, Institute of Animal Science – Kostinbrod, Bulgaria
**Corresponding author*: ijantcev@mail.bg

Abstract

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The aim of the present study was to estimate the values of some biochemical parameters - ALT, AST, ALP, Fe and glucose of two *Karakachan sheep* herds – IAS-Kostinbrod (I.) and Milanovo (M.), reared at different altitudes (740 and 1150 m above sea level, resp.) with different body condition score (BCS) and hematocrit (HCT) levels. Analysis of biochemical parameters was performed in extracted plasma with a BTS-350 Semi Automated Biochemical Analyser. The body condition score was performed during sampling.

The BCS of the sheep from the herd of I. was better than that of the animals from M., as the difference was significant for the herds overall ($p<0.05$). The HCT levels of the sheep from the I. herd were also higher (but not significantly) than those of the animals from M. The activity of ALT was higher than reference values and significantly lower in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.01$). The activities of AST and ALP were lower than reference values, as well as the levels of plasma iron and glucose. The differences were significantly higher in the sheep from M. compared to those from I. in general for the herds (AST, ALP, Fe and glucose - $p<0.001$) and in the group with low HCT (ALP - $p<0.05$, AST and Fe - $p<0.01$ and AST - $p<0.001$). In the group with a high HCT, there were no significant differences in all biochemical parameters.

Keywords: Hematocrit; biochemical parameters; altitude; BCS; Karakachan sheep

Introduction

Because of the narrow area of distribution of the *Karakachan* breed of sheep on the Balkan Peninsula, it is logical that there are few detailed and specialized studies on it. A relatively greater number of studies about the breed are related to hemoglobin polymorphism (higher hemoglobin type A has been reported) and other blood proteins in the serum to prove the genetic relationship with other local breeds, emphasis is placed on the study of productive, reproductive and biochemical indicators (Baulov, 1992, Stevanovic et al., 2015, Odjakova et al., 2022).

A very detailed up-to-dateoverview study of the *Karakachan* breed of sheep is made by Staikova et al. (2015). According to numerous literary sources cited in it, the *Karakachan* sheep and the *Karakachan* horse are Balkan endemics, and on the basis of osteological and craniological studies, the phylogenetic relationship between the European mouflon and the *Karakachan* sheep has been proven. In this sense, this breed is considered to be one of the oldest sheep breeds in

Europe and with the smallest genetic distances with the common ancestor of domestic sheep, and the *Karakachan* sheep are also the purest form of the coarse-wooled *Tsakel* type. During the breeding of the *Karakachan* sheep over the years in our country, the so-called "transhuman pastoralism" - a traditional sheep management strategy adopted by shepherds to cope with environmental stress at different altitudes during the spring-summer and autumn-winter breeding periods. In a similar previous study of ours (Moneva et al., 2016), we also investigated 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. 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.

An interesting study of the differences in hematological and biochemical parameters in *Pramenka sheep* raised at different altitudes in Bosnia and Herzegovina was done by Hrković-Porobija et al. (2019). They found that almost all of the biochemical parameters showed variations between the sampling areas that could be caused by the influence of different geographic areas and floristic composition, as well as the current health status of the examined individuals, but calculated correlative values showed significant differences for individual hematological and biochemical parameters in sheep for both investigated areas.

Stevanović et al. (2015) aimed with their research an examination of certain metabolic profile parameters of the *Karakachan sheep* blood serum, and variability of their concentrations in comparison to age and some other indigenous sheep breeds from Balkans. They found a statistically significant difference between the mean concentrations of the studied parameters in *Karakachan* sheep and other breeds in total protein (*Tsigai*, *Dubrovnik* and *Dalmatian* sheep), albumin (*Dalmatian*), calcium and inorganic phosphorus (*Tsigai*, *Dubrovnik*) and AST's (*Dalmatian, Karakachan* sheep from Bulgaria).

Bozhilova-Sakova and Dimitrova (2020) also conducted a comparative study on some of the most frequently investigated biochemical parameters of blood serum in six Bulgarian sheep breeds - *Karakachan* (KK), *Cooper-Red Shumen* (CRS), *Synthetic Population Bulgarian Milk* (SPBM), *Caucasian Merino* (CM), *Karnobat Merino* (KM) and *Il de France* (IdF). They found that ALT, AST, LDH, ALP, urea and creatinine have had a high variation of the values between the different breeds and between different parameters because they have been largely influenced by stress and the general condition of the animals.

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 assessment. 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 suspected malnutrition. On the other hand, Abdel-Lattif and Al-Muhja (2021) in their investigation with *Awassi* sheep found that the age of the animal and body weight has not affect the other blood and biochemical parameters (RBCs, PCV, Neutrophils, Basophils, Monocytes, Lymphocytes,Albumin, AST, ALT).

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 (Ouaneset al., 2011,Bezerra et al., 2017, 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 (Rahman et al., 2018,Khalil et al., 2022). 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.Rathwa et al. (2017) also found clear, reliable differences between the levels of the studied biochemical indicators, including AST, ALT, glucose, etc. in summer and winter season in indigenous sheep in Anand, India as well as Soliman (2014) found that serum ALT activity showed higher ($P<0.05$) levels in late-pregnant and non-pregnant compared to early-lactating *Ossimi* ewes with no significant difference in serum AST levels in Egypt.

In the context of the studies cited above, the aim of the present study was to estimate the values of some biochemical parameters - ALT, AST, ALP, Fe and glucose of two *Karakachan* sheep herds – IAS-Kostinbrod (I.) and Milanovo (M.), reared at different altitudes (740 and 1150 m above sea level, resp.) with different body condition score (BCS) and hematocrit (HCT) levels.

Material and Methods

The research was carried out in a sheep farm in the experimental base "Zlatusha" of the IAS - Kostinbrod (I.) -740 m above sea level) and a sheep farm in the village of Milanovo - "Govedova voda" locality, Vratsa Balkan Mountain (M.) - 1150 m above sea level, in June 2022. Both herds of Karakachan sheep (1.5-7.5 years old) were reared traditionally for the breed on pasture during the sampling period, without feeding with concentrated feed, the ewes were not milked, and the lambs nursed from them until their natural weaning. The herd shearing campaign was carried out one week prior to sampling. From all sheep in the respective herds, representative samples of 50 animals each were selected, 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).

The levels of hematocrit (HCT) in a whole blood were determinedby a 5-Part-Diff Automated Hematology Analyzer (laser multidimensional hematology analyzer) URIT-5160. Analysis of biochemical parameters after that was performed in extracted plasma from the same samples with a BTS-350 Semi Automated Biochemical Analyser, manufactured by BioSystems Ltd., Spain.

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 narrow individual variation, 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 sheep from the herd of I. was better than those from M. in general for the herds, and the difference is significant ($P<0.05$). In the low and high hematocrit groups, the trend was the same, but the differences were not significant (Fig. 1). A possible explanation for this fact is that the fertility (twinning) of the ewes from I. during the year is slightly higher, which correlates with the studies of Todorov et al. (1994), according to which ewes in good physical condition breed more easily and twin to a greater extent than lean ewes. 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 activ-

ity 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.

The hematocrit levels of the sheep from the I. herd were also higher than those of the animals from M., both for the herds as a whole and in the sample with low hematocrit, and the differences were not significant (Fig. 2). In the High hematocrit group, the sheep from M. have a slightly higher hematocrit, and the difference is also not significant. This is in contrast to the expected higher levels of hematocrit in the *Karakachan* sheep from M., 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 should 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 hematocrit 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 hemoglobin 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 of this phenomenon can be found in the above results for the Body Condition Score, which suggest a stronger metabolism in the sheep from I. for its maintenance, respectively, the need for more oxygen for metabolic processes.

Alanine aminotransferase (ALT, ALAT, SGPT) is an enzyme that belongs to the group of aminotransferases, which are responsible for glycogen synthesis. It is found mainly in the cells of the liver (hepatocytes) and in smaller amounts in

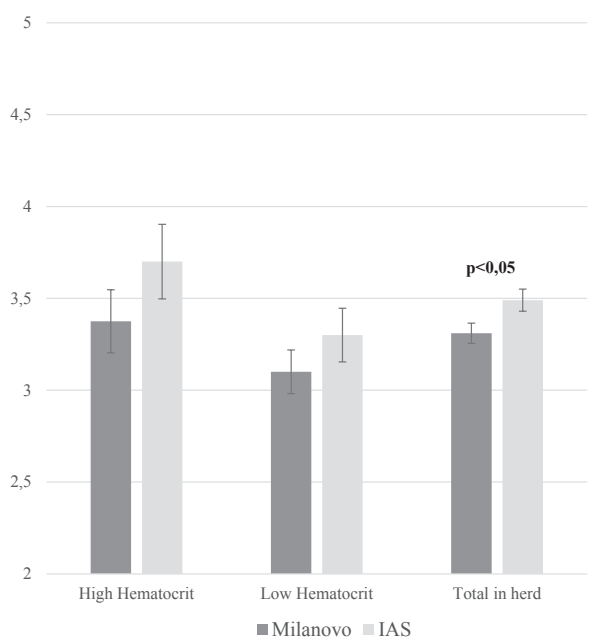

Fig. 1: BCS in two Karakachan sheep herds (range 1-5)

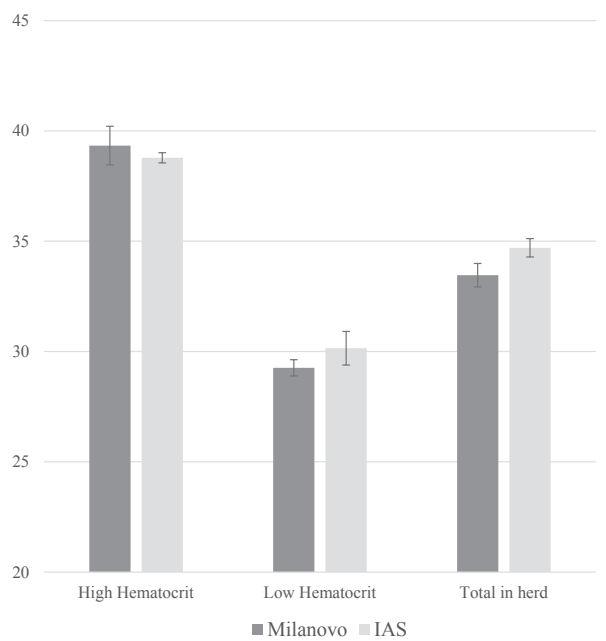

Fig. 2: HCT levels in two Karakachan sheep herds (%)

the kidneys, myocardium and skeletal muscles. Its main function is the transfer of amino groups between alpha-amino and alpha-keto acids in hepatocytes. This process is assisted by coenzyme pyridoxal phosphate. ALT is mainly located in the cytoplasm unlike AST. When hepatocytes are destroyed as a result of an infectious or toxic process, the concentration of ALT in the serum increases. ALT is also a more specific indicator of liver damage than AST. It increases even with minimal liver damage, even before the appearance of symptoms and signs of the disease (Kaneko et al., 1997).

In our research the activity of ALT (Fig. 3) in all investigated samples was between 99 and 132 U/L, which represents an increase around four times up the reference values for sheep (22-38 U/L) established by Kaneko et al. (1997) and by Radostits et al. (2000). These results are also higher than those, reported by Bozhilova-Sakova and Dimitrova (2020) - 6.3±1.41U/L and even those, reported by Angelov et al. (2013) - 25.2 l ± 6,51 U/l in their similar investigations with *Karakachan* sheep. A possible reason for that could be the period of the year as well as the stress effect of shearing. The activity of ALT was significantly lower in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.01$). In the group with a high HCT there were no significant differences.

However, unlike ALT, which is found almost entirely only in the liver, aspartate aminotransferase (AST, ASAT, GOT) can be found in other places in the body - the heart muscle, skeletal muscles, kidneys, pancreas, brain tissue, spleen. This makes it a less specific indicator of liver function than ALT or other indicators (Kaneko et al., 1997). AST values above the upper reference limits (from 10 to 100 times the norm) are usually observed in conditions that are accompanied by acute hepatic necrosis - viral hepatitis, toxic hepatitis, tetrachloromethane poisoning. A rapid rise and then fall in AST values may be associated with extrahepatic biliary obstruction. A decrease in AST values can be a consequence of azotemia (increased levels of nitrogen-containing substances in the blood), chronic kidney dis-

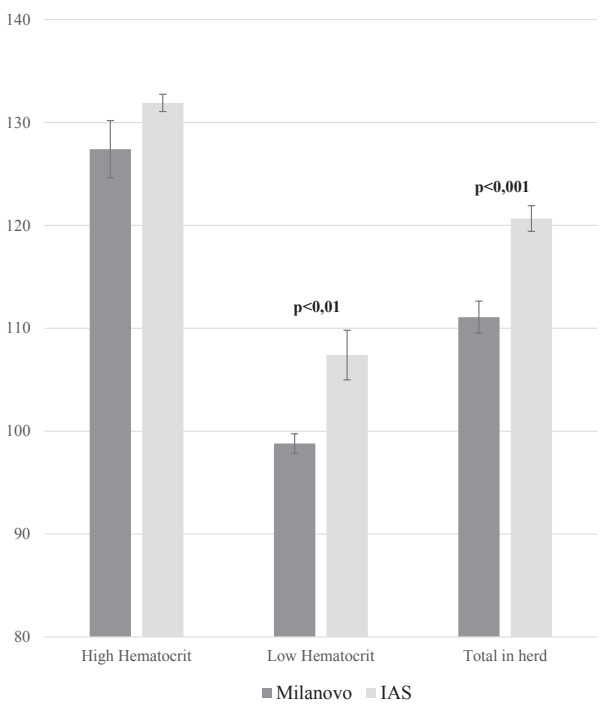

Fig. 3: ALT levels in two Karakachan sheep herds (U/L)

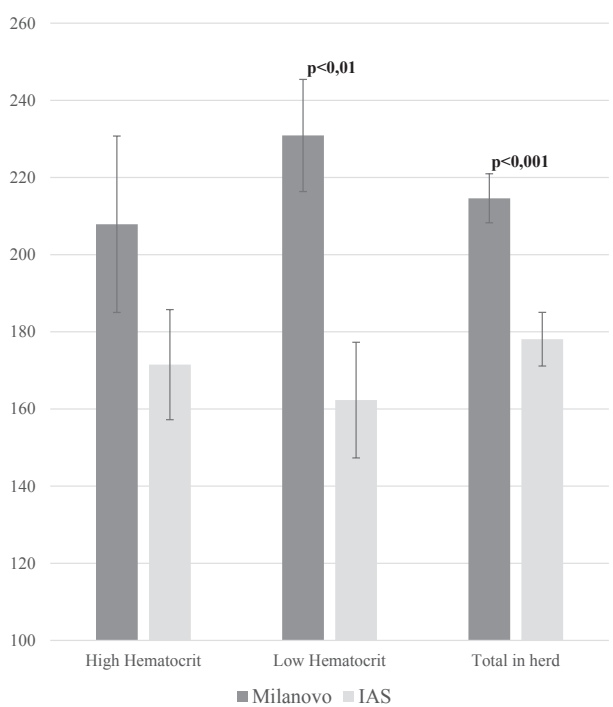

Fig. 4: AST levels in two Karakachan sheep herds (U/L)

ease and in patients on long-term dialysis, vitamin B_6 deficiency in chronic alcohol abuse and malnutrition (undernourishment). A low level of AST has no clinical significance.

The activities of AST (Fig. 4) in our study were between 162 and 231 U/L, which was close to the reference values for sheep - 60-280 U/L (Kaneko et al., 1997), Radostits et al., 2000). Stevanović et al. (2015) found that the mean concentration of AST in *Karakachan* sheep blood serum in Serbia (97,66±7,72 U/L) have been significantly lower ($p < 0.001$) compared to the activity of this enzyme in the *Dalmatian* breed. The obtained values of AST in *Karakachan* sheep from Bulgaria in different studies were 121.29 ± 32.35 U/L (Angelov et al., 2013) and 87±24.90 U/L (Bozhilova-Sakova and Dimitrova, 2020). The differences were significantly higher in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.01$). In the group with a high HCT, there were no significant differences in all biochemical parameters again.

Alkaline phosphatase (ALP) is an enzyme that occurs in the body as four subtypes - hepatic, bone, renal and placental during pregnancy. ALP is a zinc-containing metalloenzyme that is activated by Mg^{2+} and other divalent ions. Alkaline phosphatase is an enzyme - hydrolase that catalyzes the hydrolysis of various phosphate esters with a pH-optimum in the alkaline range. This enzyme has different isoforms, some of which are true isoenzymes, ie. encoded by different genes. ALP is widely distributed in various tissues of the body, being especially associated with bone (osteoblasts), small intestine (mucosal cells), liver (cells of the biliary system), placenta and kidney (proximal tubules). In bones, ALP is associated with mineralization, possibly by catalyzing the formation of phosphates from pyrophosphates. In the intestine, there is evidence that it is involved in the transport of lipids. Intra-individual variation of serum ALP was 25.4%; inter-individual variations – 6.7%, and the sources of variations can be observed mainly in zinc deficiency (Kaneko et al., 1997).

In our study the activity of ALP (Fig. 5) was between 25 and 74 U/L, which represents a small decrease comparing the reference values for sheep (70-390 U/L) set by Kaneko et al. (1997) and by Radostits et al. (2000). These results are also lower than those, reported by Bozhilova-Sakova and Dimitrova (2020) - 222.45±101.08U/L and those, reported by Angelov et al. (2013) - 192,94 ±36,51 U/l in their similar investigations with *Karakachan* sheep. Absolutely the same differences and trends like about ALT and AST were observed about ALP – there were significantly higher in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.05$) and in the group with a high HCT there were no significant differences in all biochemical parameters.

Iron is an ion, which in the largest percentage is contained in hemoglobin and myoglobin. Very small amounts of it are also contained in some enzymes. In the body, unbound iron is stored in the form of ferritin and hemosiderin. In the blood, it binds to the transport protein transferrin (Kaneko et al., 1997). Due to impossibility of excretion of iron from the body, its concentration is strictly regulated. Iron is transported in combination with carrier proteins. These are transferrin, lactoferrin and mobilferin-1. It should be noted that the amount of se-

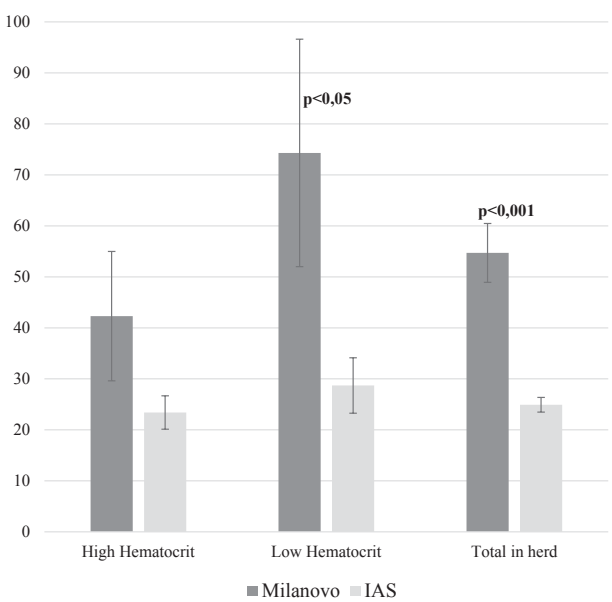

Fig. 5: ALP levels in two Karakachan sheep herds (U/L)

rum iron is an indicator that varies depending on the day and even the time of day. To minimize inaccuracies, determining the level of iron in the blood is usually combined with other tests. These include a total serum iron-binding capacity (TIBC) assay, as well as ferritin and transferrin assays (Braunet al., 2010)

We found low levels of plasma iron – between 10 and 17 μmol/L (Fig. 6) according the reference values (30-40 μmol/L) set by Radostits et al. (2000). On the other hand we observed normal HCT and HGB levels in the same samples, which could be related to the negative feedback mechanism as well. Again there were significantly higher levels of Fe in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.01$) and in the group with a high HCT there were no significant differences.

The two hormones that directly regulate blood glucose levels are glucagon and insulin. Glucagon accelerates the conversion of glycogen into glucose and thus raises blood sugar. Insulin helps glucose enter cells, stimulates glycogen formation and lowers blood sugar levels. Other hormones that play an important role in glucose metabolism are: adrenocorticotropic hormone (ACTH), glucocorticoids, adrenaline, thyroxine. They all raise blood glucose levels, while only insulin lowers it(Kaneko et al., 1997).

Glucose levels in almost all investigated samples were normal (Fig. 7) according the reference values (1.7-3.6 mmol/L) set by Radostits et al. (2000). The only exception with low glucose levels (0.86±0.32 mmol/L) we observed in low HCT group of sheep from I. There were significantly higher levels of glucose in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.001$) and in the group with a high HCT there were no significant differences again.

In conclusion, we can note that the biochemical 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 Body Condition Score of the studied herds from the same breed. Our assumption was that the increased metabolism (for increased BCS and glycogen synthesis, respectively) in the sheep from the IAS perhaps in all likelihood compensated the higher altitude in the animals

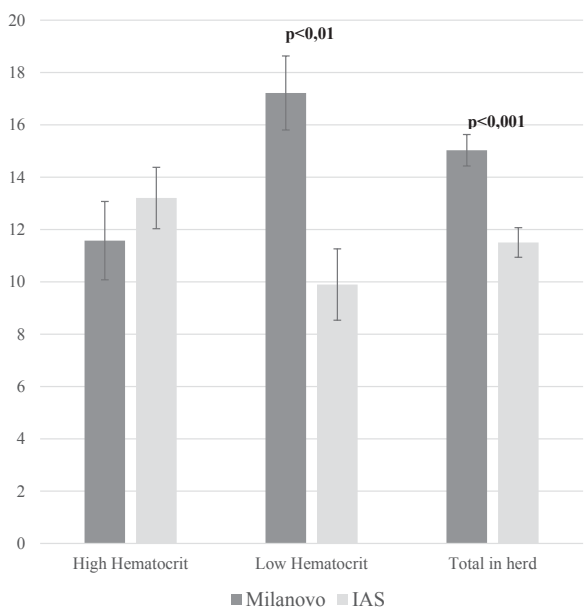

Fig. 6: Fe levels in two Karakachan sheep herds (μmol/L)

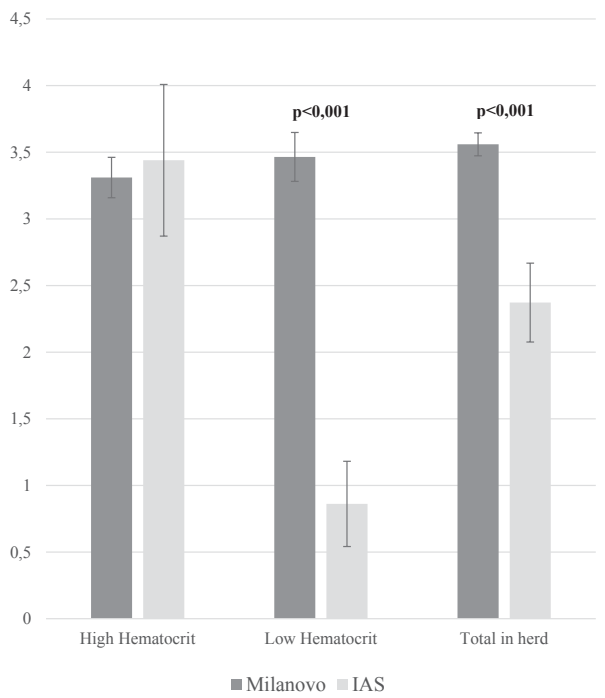

Fig. 7: Glucose levels in two Karakachan sheep herds (mmol/L)

reared in the village of Milanovo but sheep with high HCT levels in both herds demonstrated low variability in all investigated samples.

Conclusions

The Body Condition Score of the sheep from the herd of IAS – Kostinbrod (I.), was better than that of the animals from the village of Milanovo (M.), as the difference is significant for the herds overall ($P<0.05$). There were no significant differences between the mean values of the two herds, as well as those of the low and high hematocrit groups about the levels of hematocrit (HCT);

The activity of ALT was higher than reference values and significantly lower in the sheep from M. compared to those from I. in general for the herds ($p<0.001$) and in the group with low HCT ($p<0.01$). The activities of AST and ALP were lower than reference values, as well as the levels of plasma iron and glucose;

The differences were significantly higher in the sheep from M. compared to those from I. in general for the herds (AST, ALP, Fe and glucose - $p<0.001$) and in the group with low HCT (ALP - $p<0.05$, AST and Fe - $p<0.01$ and AST - $p<0.001$). In the group with a high HCT, there were no significant differences in all biochemical parameters.

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