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THE IMPACT OF PHYSIOLOGICAL CONDITION ON CALCIUM, PHOSPHORUS AND MAGNESIUM LEVELS IN SHEEP BLOOD SERUM

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Abstract: A large number of sheep, especially the ones that spend the majority of time on pastures, is being fed with the meals which do not contain all the neccessary mineral substances. The level of calcium, phosphorus and magnesium on natural pastures is too low considering the neccessities of sheep. So, the irregularities in sheep feeding occur because of the absence of other food sources. These irregularities range from the acute mineral deficite or illness, to the mild temporary forms which can hardly be diagnosed, but they affect the level of production. The contents of calcium, phosphorus and magnesium in sheep blood serum are given in this paper, as some of the indicators of the need for supplementation of these substances. The average calcium level in the blood serum of the control group was 2.61 mmol/l, phosphorus level was 1.04 mmol/l and magnesium level was 1.31 mmol/l. The average calcium level in the blood serum of the tested group was 2.33 mmol/l, phosphorus level was 0.92 mmol/l and magnesium level was 1.37 mmol/l.

Key words: sheep, calcium, phosphorus, magnesium, serum

Introduction

The knowledge of the nutritive values of the grass and hay mix, with which sheep are fed during nearly one half of the year, is very important from the standpoint of the evaluation of food quality. Pasture is very important in sheep feeding as it is the full-value source of food, which could not be easily substituted when considering the extensive breeding of ruminants. The quality of the pasture and its nutritional value depend on many factors, but primarily on the quality of the soil and the climatic factors. While breeding sheep in the Šar Mountains, learning about the quality of pastures gave us the possibility of taking appropriate measures for improving the existing conditions. That was the starting point for resolving

issues of proper sheep nutrition during summer, because that period represents an important phase of a physiological change that occurs in sheep (lactation, and preparation for successful impregnation).

Certainly, these tests are not able to discover the genesis of these conditions, which could give us guidance for further development by applying Hemo - Agro - Hido and other measures, all dictated by the ecological factors. Because these researches do not have pretensions of such a capacity and profile, their goal is to give representation of grass chemical composition of the tested pastures, which is a very useful component of the main research, and is also related to the dynamics of calcium, phosphorus and magnesium in stages of vegetation, or the exploitation of the pastures. In many areas of the Šar Mountains, the content of stodgy cattle food is not tested enough. From the results obtained on the basis of our previous researches of mineral substances in stodgy food, a low content of phosphorus is detected, which was not frequently the case with calcium. This phenomenon is especially present during dry years and certain periods which often occur at the Šar Mountains where the testing was performed. It should be added that at the Šar Mountains, there are lands in which the content of physiologically active phosphorus (P_2O_5) is extremely low, as was found by *Mirić (2000)*, and a low content of this element in the soil adversely affects its content in plants. To this we should add the statement of *Stojković (2006)* that a substantial number of plant species from these areas has a low content of phosphorus.

Materials and Methods

The testing of the influence of the physiological conditions and the seasons on the dynamics of the content of calcium, magnesium and phosphorus in the blood serum of the Zapata sheep in Štrpce was conducted in 2008/2009. There were 100 sheep in the experiment, 50 of which were in the control group – the futile sheep (treatment 1, 2, 3, 4 and 5) and 30 in the experimental group - the fertile sheep (treatment 1, 2, 3, 4 and 5). At the end of each treatment, blood was taken from the jugular vein of the sheep for the serum preparation. The testing of these mineral elements was carried out in the sheep blood serum and the food used in their meals. The sheep were staying in a cottage at the Šar Mountains from the late May until the early October (2008). The main meal was their pasture with the addition of 200 g of corn grits. And from October until May (2009), the sheep were in their home environment near Štrpce. The main meal was their meadow hay from 1.5 to 2.0 kg and corn grits from 200 to 300 g per day. The cattle salt in the form of the mineral plates was also available to the sheep.

Statistical analysis was performed using Statistica, version 6, *SatSoft. Inc. (2003)*.

The level of calcium, phosphorus and magnesium in the blood serum of sheep and the food which is their meals is examined. The blood was taken from the

sheep from the v. jugularis and the blood serum in which the level of these minerals was tested was insulated from it. In these samples of feed, calcium is examined with the permananganate method, and in the samples of the blood serum atomic-absorption method, concave cathode lamp HCL at the wave length of 422.7 nanometers. In the samples of feed, phosphorus is examined by the conventional gravimetrical method, measured in the form of $Mg_2P_2O_7$, and in the samples of the serum by colorimetrical method on the photo clorimeter. In the feed, magnesium is examined by the gravimetrical method in the form of MgO, and in the serum at the atomic absorber.

Table 1. The contents of nutrients in the feed for feeding sheep

Type of food	Chemical composition, (%)						
	Water	Protein	Cellulose	Ash	Ca/g	P/g	Mg/g
Meadow hay	11.15	8.37	30.67	7.80	0.76	0.26	0.20
Lucerne hay	11.90	9.80	28.90	8.58	0.98	0.15	0.34
Corn grits	11.51	8.51	1.80	1.56	0.10	0.35	0.19
Spring grass	20.71	9.67	24.90	6.79	0.76	0.15	0.05
Summer grass	18.68	6.80	30.22	5.56	0.77	0.16	0.06
Autumn grass	18.12	5.65	28.40	8.23	0.78	0.30	0.08

Results and Discussion

The data about the dynamic of calcium content in the sheep blood serum are shown in table 2. After the fertilization of sheep, the content of calcium in blood serum was 2.54 mmol/l, while it was 2.74 mmol/l for the infertile sheep at that time. In the second half of the pregnancy, the content of calcium decreased to 2.34 mmol/l, while in infertile sheep this decrease was much lesser (2.59 mmol/l). In the phase of the most opulent secretion of the milk in the lactating sheep, the decrease in the content of calcium is most pronounced, and it was slightly below the physiological limits (2.02 mmol / l). At that time, the content of calcium in the blood serum of the infertile sheep was at the same level (2.58 mmol / l). In the phase of reduced milk secretion in the lactating sheep, the increase in the content of calcium of 4.00% was noted, and later after the lactation period, the level of calcium in the blood serum climbed to 2.50 mmol / l. At that time, in the infertile sheep, the content of calcium remained at the same level (2.57 and 2.60 mmol/l). Considering the dynamics of the content of calcium in the blood serum of fertile and infertile sheep, the general conclusion can be made, that, normocalcemia existed at the conception of the sheep and that the changes, that occurred in the fertile sheep as the result of normal moving, were caused by the different needs of the organism in accordance with the physiological conditions. Considering the content of calcium in the blood serum of the sheep it can be concluded that

calcemia was normal and that there were no significant differences (P>0.05) in physiological conditions, or season.

Table 2. The calcium contents in sheep blood serum (mmol/l)

The of taking blood	n	X±SD	SG
Control group – infertile sheep	50		
Tretment 1 - August	10	2.47±0.18	0.47
Tretmenrt 2 - November	10	2.59±0.18	0.47
Tretment 3 - January	10	2.58±0.13	0.35
Tretment 4 - July	10	2.57±0.24	0.64
Tretment 5 - September	10	2.60±0.17	0.44
Average	10	2.61±0.19	0.47
Tested group – the fetile sheep	50		
Tretment 1 – first half of the pregnancy	10	2.54±0.25	1.05
Tretment 2 – second half of he pregnancy	10	2.34±0.11	0.45
Tretmen 3 - the beginning of lactation	10	2.02±0.13	0.54
Tretment 4 – the end of lactation	10	2.29±0.15	0.60
Tretment 5 – the period of becoming infertile	10	2.50±0.11	0.47
Average	10	2.33±0.15	0.62

The dynamics of the content of calcium in the blood serum shows a regular rhythm when we correlate it with changes in the physiological status of the sheep, making a concave parabola with the lowest point in the time of the most opulent secretion of milk. At the same time, the contents of calcium in the serum in the futile sheep ranged slightly above average the normal values (between 2.57 and 2.74 mmol / l) and despite the season it was not subjected to changes. The percentage of variation in the content of calcium was significantly lower in the futile sheep, which is understandable.

According to the data from *Mirić (2000)*, this value was lower for 5.15% of the average content of calcium in our previous experiments, *Stojković (2006, 2009)*. But, in literature there are the data according to which the content of calcium in the blood serum of sheep can be much higher, *Pavličević et al. (1998)*, as was the case when the minerals with high calcium content were added to the meals. But there are also data on cases of the expressive hypocalcimia such are indicated by *Mirić (2000)* in whose experiments the level of calcium in the blood reach the level of only 1.98 mmol /l.

The data about the content of phosphorus in the blood serum of the sheep are shown in the table 3. In the first half of the pregnancy, the content of phosphorus in sheep blood serum was 1.13 mmol/l. in the second trimester the content decreased to 0.86 mmol/l. During the period of full lactation, the maximum decrease was 0.74 mmol/l. From this, along with the decrease in the secretion of the milk, the level of phosphorus increased to 0.92 mmol/l, only to reach the level

of 0,97 mmol/l in the infertile sheep in September. In the infertile sheep, the content of phosphorus in the blood serum in August was 0.98 mmol / l and it was constantly increasing during the year (1.01 mmol / l, 1.02 mmol / l, 1.07 mmol / l and 1.133 mmol / l) to reach in the beginning of September the level of 1.13 mmol / l. In the fertile sheep, the smallest deviation from the average values between the certain sheep was noticed after the fertilization and this deviation was constantly growing from the period of becoming infertile. In terms of variations of the percentage of phosphorus content in infertile sheep blood, the procedure was reversed. The variations were the highest at the time when sheep were impregnated and they were constantly declining until September.

Table 3. The phosphorus content in sheep blood serum (mmol/l)

The of taking blood	n	X±SD	SG
Control group – infertile sheep	50		
Tretment 1 - August	10	0.98±0.16	0.43
Tretmenrt 2 - November	10	1.01±0.15	0.39
Tretment 3 - January	10	1.02±0.08	0.22
Tretment 4 - July	10	1.07±0.06	0.15
Tretment 5 - September	10	1.13±0.04	0.11
Average	10	1.04±0.09	0.26
Tested group – the fertile sheep	50		
Tretment 1 – first half of the pregnancy	10	1.13±0.15	0.62
Tretment 2 – second half of the pregnancy	10	0.86±0.09	0.37
Tretmen 3 - the beginning of lactation	10	0.74±0.08	0.33
Tretment 4 – the end of lactation	10	0.92±0.08	0.35
Tretment 5 – the period of becoming infertile	10	0.97±0.19	0.40
Average	10	0.92±0.10	0.41

The presented data show that the content of phosphorus in sheep blood serum during the year is, in all physiological states, under the physiologically expected velues. Hypophosphathimia reached its peak in the fertile sheep in the phase of the most opulent secretion of the milk. Furthermore, hypophosphathimia was very pronounced in the fertile sheep, so the average variations of the inorganic serum phosphorus during the year were higher than in infertile sheep. Differences in the content of phosphorus per season and physiological condition were statistically significant ($P < 0.01$).

The content of phosphorus for all groups in the experiment was in the average of 0.98 mmol/l of the serum. With these values it was located below the physiologically normal ranges of the content according to the data from the literature (1,45-2,00 mmol / l).

The lowest values were recorded in the most opulent stage of lactation (0.74 mmol / l) and the highest in the first half of the pregnancy (1.13 mmol /

l). With lactation passing off and the arrival of the nonproductive lactation period (in autumn), the content increased to 0.97 mmol / l. In terms of variation, it is characteristic that, from the beginning to the end of pregnancy in the monitored period, the variety grew more and more (from 5.03 to 15.50%). Although, in infertile sheep, the average content of phosphorus in the serum was higher than in the fertile sheep by 11%, the general level was very low, and the changes were more subordinate to the factor of diet, which was not the case in the fertile sheep. The content of phosphorus in the infertile sheep was constantly increasing during the year, starting from 0.98 mmol / l in August to 1.13 mmol / l at the end of the observed period. Concerning the content of phosphorus in the serum of the fertile and infertile sheep, it can be concluded that hypophosphatemia is evident during the whole year.

During the general hypophosphatemia, of both fertile and infertile sheep, it is important to notice the appearance of a very different type of variation in the content of phosphorus per year, namely caused by the physiological state. In the infertile sheep it can be seen as a constant, although small increase in the content of phosphorus during the "rest period" until the next season of fertilization, with the simultaneous narrowing of the variation span, whereas in the impregnated sheep, we have a decrease in the content of phosphorus, which results from the prolonged exposure of organism to stress (increased distress) in pregnancy in the first two months of lactation, and its increase in the period of physiological relief of the organism, with a permanent increase in the percentage of the variations in the blood serum phosphorus content. These findings support the assumption that in the state of hypophosphatemia in the temporary infertile sheep, the balance of phosphorus is gradually improving to such an extent that they can, after a one-year break, manifest the need for breeding and be fertilized. In the "fertile" sheep, although the balance of phosphorus in the second half of the lactation mainly improves, it can not be improved in all animals, which is attested by the increased percentage of variation so that slightly more than a half of the sheep came to a successful fertilization, while a smaller part remained unfertilized.

We can see, from the displayed value of phosphorus content, that there was a low level of this element in the sheep blood serum with a minimal increase in the time of pregnancy, with the lowest value in the stage of lactation. The reason for such a low content of phosphorus in sheep blood is seen in its low content in food, especially hay, and the reason for the low content in hay, according to *Mirić (2000),* lies in the fact that there are large areas of land in this region in which the content of physiologically active phosphorus (P_2O_5) is extremely low. This is confirmed by the findings of *Stojković (2006, 2009)* which demonstrate that a large number of plant species from these areas have a low content of phosphorus. A particularly low content of phosphorus in sheep blood serum in lactation period can be interpreted by its enhanced secretion in milk.

The data about the dynamics of the content of magnesium in the blood serum of the sheep are shown in the table 4.

The content of magnesium in sheep blood serum was within the normal range and it showed no significant differences with changes in the physiological state of the sheep, which is its characteristic behavior. Similar or the same range of magnesium levels in the blood was also established for the same intervals in the infertile sheep. Differences in the magnesium content in sheep blood serum, related to both season and the physiological conditions were not significant (P> 0.05).

The magnesium content in sheep blood serum of was within the normal physiological range of 1.34 mmol / l and showed no significant differences during the changes in the physiological state, which is its characteristic behavior. Although to a lesser extent, there was a reduction of its content in sheep blood at the end of lactation (1.27 mmol /l). There were not any notable individual changes in the magnesium content, or changes in groups of sheep with different lactation, but averages per groups indicate the existence of a reverse trend of a change in the content of this element in the blood serum in relation to the trend of a change in the level of calcium and phosphorus, which means that the content of magnesium was the highest in the blood serum of the sheep with the highest lactation, and the lowest in the infertile sheep.

Table 4. The magnesium content in sheep blood serum (mmol/l)

The of taking blood	n	X±SD	SG
Control group – infertile sheep	50		
Tretment 1 - August	10	1.28±0.12	0.27
Tretmenrt 2 - November	10	1.32±0.17	0.19
Tretment 3 - January	10	1.29±0.19	0.16
Tretment 4 - July	10	1.35±0.11	0.21
Tretment 5 - September	10	1.35±0.23	0.10
Average	10	1.31±0.16	0.18
Tested group – the fertile sheep	50		
Tretment 1 – first half of the pregnancy	10	1.33±0.15	0.62
Tretment 2 – second half of he pregnancy	10	1.31±0.21	0.37
Tretmen 3 - the beginning of lactation	10	1.43±0.23	0.40
Tretment 4 – the end of lactation	10	1.27±0.10	0.59
Tretment 5 – the period of becoming infertile	10	1.55±0.18	0.61
Average	10	1.37±0.17	0.51

The data on the content of magnesium, which were obtained in these researches, correspond with the results of *Adamović and Pavličević (1990)* and *Pavličević et al. (1999)*. The explanation for the relatively small changes in magnesium content in sheep blood, according to *Stojkovic (2006, 2009)*, is that the

magnesium in soft tissues is not reduced even when the sheep loses up to 30% of magnesium from the skeleton.

Conclusion

The content of calcium, phosphorus and magnesium in sheep blood serum was investigated as one of the indicators of the need for supplementation of these materials to animals. The average content of calcium in the examined sheep blood serum was 2.47 mmol / l, of phosphorus was 0.98 mmol / l and of magnesium was 1.34 mmol / l of the serum. The values of calcium were at the upper limit of normal content referred to in the literature. The changes in the content of calcium were not significantly manifested in relation to the season and the physiological state of the sheep. The values of phosphorus were below the deficit, and these values decreased mostly in the beginning of the sheep lactation period. The content of magnesium was within the normal physiological range. The changes in the content of magnesium were not significantly manifested in relation to the season and the physiological state of the sheep, but had a reverse trend compared to the trend of change in calcium and phosphorus.

Uticaj fiziološkog stanja na sadržaj kalcijuma, fosfora i magnezijuma u krvnom serumu ovaca

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Rezime

Veliki broj ovaca, naročito onih koji dobar deo vremena provode na paši, hrani se obrocima koji ne sadrže sve potrebne mineralne materije. Nivo kalcijuma, fosfora i magnezijuma na prirodnim pašnjacima je suviše nizak u odnosu na potrebe ovaca. Tako se nepravilnost u ishrani javlja kod ovaca u odsutnosti drugih izvora hrane. Ove nepravilnosti kreću se od akutnog mineralnog deficita ili bolesti, pa do blagih prelaznih formi koje se teško dijagnosticiraju, ali se odražavaju na nivo proizvodnje. U radu je dat sadržaj kalcijuma, fosfora i magnezijuma u krvnom serumu ovaca, kao jednog od indikatora obezbeđenosti ovaca ovim materijama. Prosečan sadržaj kalcijuma u krvnom serumu kontrolne grupe ovaca iznosio je 2,61 mmol/l, fosfora 1,04 mmol/l i magnezijuma 1,31 mmol/l seruma. Kod ogledne grupe prosečan sadržaj kalcijuma bio je 2,33 mmol/l, fosfora 0,92 mmol/ i magnezijuma 1,37 mmol/l seruma.

References

ADAMOVIĆ M., PAVLIČEVIĆ A. (1990): Zastupljenost kalcijuma, fosfora i magnezijuma u hrani za telad i mogućnost podmirivanja potreba. Inovacije u stočarstvu, IX, 99-105.
BONČEV S. (1989): Soderžanie Ca, P, Mg, Na, Fe i Cu v sivorotke krovi ovec v zavisimot od fiziologičesgo sostojania. Životnodstv. Nauki, 21, No 6.
MIRIĆ M. (2000): Krmna baza Kosova, mon.
NRC (1985): Nutrient requirements of sheep. Sixth Revised Edition.
ODINEC R.N., ASANBEKOV O.A. (1986): Mikroelementi v životnovodstve i rastenievodstva, Frunze, ILIM.
PAVLIČEVIĆ A., KRAJINOVIĆ M., SAVIĆ D. (1994): Aktuelna neadekvatna mineralna ishrana preživara i mogućnost saniranja. Biotehnologija u stočarstvu, 10, 1-2, 109-115.
STOJKOVIĆ J., RAJIĆ I., RADOVANOVIĆ T. (1996): Pregled i ocena stočne hrane, mon. Novi svet. Priština.
STOJKOVIĆ J., JAŠOVIĆ B. (2006): Mineralna hraniva u ishrani domaćih životinja. XI savetovanje o biotehnologiji, zb. radova 411-419, knjiga 2. Agronomski fakultet, Čačak.
STOJKOVIĆ J. (2009): The contents of some mineral elements in the blood serum of sheep in different physiological cycales and with the different level of minerals a meal. Bioteshnology in animal husbandry, 25, 5-6, 978-984.
STATISTICA, Version 6, StatSoft, Inc. 2003.

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