

ANALYSIS OF SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS IN THE BLOOD OF DAIRY SHEEP IN TWO DIFFERENT SAMPLING AREAS

Amina Hrković-Porobija¹, Aida Hodžić¹, Husein Ohran¹, Alma Rustempašić²,
Lejla Velić¹, Almira Softić¹, Kenan Baltić¹, Kenan Podžo¹

Original scientific paper

Summary

This study aimed to investigate the concentrations of cholesterol and triglycerides in the serum of sheep from the area of Livanjsko Polje and Mount Vlasic. A total of 117 sheep of the breed pramenka were included in the research. The sheep were divided into groups according to the sampling area through different periods (July, August, and September). Using an automatic analyzer (Kodak Ektachem analyzer DT 60 II, DTSC Module, and DTE Module), the concentrations of cholesterol and triglycerides were determined in the serum of sheep. In the area of Livanjsko Polje, the highest established value of cholesterol in sheep serum was in the second sampling period, with statistical significance compared to the other two sampling periods. Cholesterol values in the Vlasic Mountain area were within the physiological range. The highest established triglyceride concentration in the area of Livanjsko Polje was also the second sampling period, with statistical significance ($p < 0.05$) compared to other sampling periods, as well as the same period from the Travnik area. The results of triglycerides in the blood serum of sheep from the Vlasic mountain area show a tendency to decrease in the third sampling period, at the end of the lactation period. These results may be due to the effects of various factors, such as age, gender, health, lactation status, and sheep breed.

Keywords: *sheep, serum, cholesterol, triglyceride*

INTRODUCTION

Blood is an important and reliable medium for assessing the health status of individual animals. Variations in blood parameters of animals are due to several factors such as altitude, feeding level, age, sex, breed, diurnal and seasonal variation, temperature, and physiological status of animals. Serum biochemical tests are widely used for the diagnosis of serious animal diseases which can lead to economic losses in animals like reduced fur, wool, and milk production (Kiran *et al.*, 2012). Ruminants present many nutritional, physiological, and metabolic peculiarities in comparison to monogastric

¹ University of Sarajevo, Veterinary faculty of Sarajevo, Department of Chemistry, Biochemistry and Physiology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina

² University of Sarajevo, Faculty of Agriculture, Department of Animal Breeding, University of Sarajevo, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina

Corresponding author: Amina Hrković-Porobija (amina.hrkovic@vfs.unsa.ba)

animals. In particular, in sheep and other ruminants, the plasma lipid profile is characterized by low triglyceride and triglyceride-rich lipoprotein concentrations. This is in agreement with species showing weak participation of the dietary lipids to the lipemia and showing the minor hepatic synthesis of fatty acids (Mazur *et al.*, 2009). The production and reproduction performance of an animal depends not only on the flow of energy, food, and water within the animal's biological system but also on its adaptability to the environment in which it lives. The variations in environmental variables such as ambient temperature, relative humidity, wind, and rainfall, have been recognized as potential hazards in livestock growth and production (Cerutti *et al.*, 2018). Serum biochemistry is important for the diagnosis, treatment, and prognosis of diseases. Determination of metabolic blood profiles, including biochemical parameters, is very important for the evaluation of the normal physiological status of animals and in helping the evaluation of management practices, nutrition, and health status (Opara *et al.*, 2010). In sheep and other ruminants, cholesterol is naturally produced in the liver and intestinal walls. It is also a source of energy and is a precursor of steroid hormones, bile acids and it is also required for normal cell function (Khan *et al.*, 2013). It plays a vital role in the formation of cell membranes, the production of hormones, bile, and metabolism of fat-soluble vitamins, acting as an antioxidant (Okonkwo *et al.*, 2010). Cholesterol is essential for the proper functioning of the body, but it can be harmful when it is consumed or produced in the body in excessive amounts. Coronary heart disease occurs when cholesterol is deposited inside the walls of coronary arteries (Khan *et al.*, 2013). Cholesterol is synthesized as a response to the energy metabolism in the liver and is generated from acetyl-CoA throughout a complex multistep process. Nutritional status and composition of dietary fatty acids seem to be a key condition to the levels of cholesterol in the blood (Mugabe *et al.*, 2017). Pregnancy and lactation modify metabolism in animals (Iriadam, 2007). Blood biochemical parameters including total protein, triglycerides, free fatty acids, and urea are important indicators of the metabolic activity in lactating animals (Karapehlivan *et al.*, 2007). During pregnancy, maternal tissues are involved in providing energy for reproduction processes, which may affect blood serum biochemistry values, affected also by several other factors as breed, age, malnutrition, fetal growth, or season (Yokus *et al.*, 2006). In sheep, during late pregnancy, blood serum lipids profile is characterized by an increased concentration of total cholesterol, triglycerides, and lipoproteins (Schlumbohm *et al.*, 1997).

MATERIALS AND METHODS

Blood sampling was performed on pramenka sheep breed in both areas during three periods: July – first period; August – second period; and September – third period. Blood samples were taken by puncture of the *v. jugularis externa* in BD Vacutainer tubes for the serum of 6 ml. Animals were tagged with an appropriate ear tag, which ensured that we always sampled the same animals over different periods. A total of

117 sheep were investigated, of which 57 in Livno and 60 in the Vlašić area. Using spectrophotometry on an automatic analyzer (Kodak Ektachem analyzer DT 60 II, DTSC Module, and DTE Module) in sheep blood serum, we determined the concentration of cholesterol and triglycerides.

Sheep diets were based on summer grazing in the presence of a large number of legumes, grasses, and herbs. The skeleton type of land with shallow arable was predominant at the investigated Livno field sites, which significantly affects the vegetation development in these areas. The floristic plantation at the site of the Vlasic Mountain ecosystem does not oscillate significantly, and species of *Nardus stricta* grasses and grasses of the genus *Festuca* have been identified, which indicates a certain acidity of the soil as these plants inhabit soils with highly acidic reactions. Statistical data processing was performed using SPSS 15.00 software. Differences were considered statistically significant at the $p < 0.05$ level.

RESULTS AND DISCUSSION

In the area of Livanjsko Polje, the highest established value of cholesterol in sheep serum was in the second sampling period, with statistical significance compared to the other two sampling periods, but also the same sampling period in the Travnik area (Table 1). If we compare the results of our analyzes from the Livno area with the values of 1.35 to 1.97 mmol reported by Kaneko *et al.* (1997), we can observe that cholesterol varies with sampling periods. The variation in results can be attributed to nutrition as a key factor in the value of cholesterolemia. Cholesterol values in the Vlasic Mountain range were within the physiological range. The sheep serum cholesterol level is significantly influenced by the physiological condition of the animal. Cholesterol concentrations are higher in pregnant animals compared to lactating sheep (Antunović *et al.*, 2002).

Table 1. The concentrations of cholesterol in the serum of sheep from the area of Livanjsko Polje and Mount Vlašić

Area	Number	I sampling period (mmol/l)	II sampling period (mmol/l)	III sampling period (mmol/l)
Livanjsko polje	57	1,97±0,11 ^{a*}	2,47 ± 0,08 ^{b*}	1,77±0,06 ^a
Mount Vlašić	60	1,09±0,11 ^a	1,71±0,08 ^b	1,1±0,06 ^b

All values represent $XX \pm Sx$. I, II, III represent sampling periods: July, August, and September. a, b = values within a sampling area with different letters are statistically significant ($p < 0,05$). * = statistically significant ($p < 0,05$) between same periods of sampling in different areas.

As a parameter of metabolism, triglycerides are a relatively variable factor. The highest concentration of triglycerides was found in the area of Livanjsko Polje in the second sampling period, with statistical significance ($p < 0.05$) compared to other sampling periods, as well as for the same period from the Travnik area (Table 2.) Triglyceride results obtained in blood from the Vlasic Mountain area show a tendency to decrease in the third sampling period or towards the end of the lactation period. Nazifi *et al.* (2003) found a significant effect of the lactation period on serum lipids and cholesterol concentration in Iranian sheep lipoproteins. With the prolongation of the lactation period, triglycerides and cholesterol levels decrease in lipoproteins. Comparing the triglyceride levels between winter and summer periods revealed a significant difference in the concentration of this parameter in winter compared to summer, which can be explained by the large influence of winter diet, regardless of breed type (Ašimović, 2005). Studies on cholesterol, triglyceride, and lipoproteins in domestic animals have made it clear that species variations exist, and that even within species, significant differences occur.

Table 2. The concentrations of triglycerides in the serum of sheep from the area of Livanjsko Polje and Mount Vlašić

Area	Number	I sampling period (mmol/l)	II sampling period (mmol/l)	III sampling period (mmol/l)
Livanjsko polje	57	0,302±0,015 ^a	0,351±0,013 ^{b*}	0,276±0,015 ^a
Mount Vlašić	60	0,316±0,016 ^a	0,262±0,014 ^a	0,312±0,016 ^a

All values represent $XX \pm Sx$. I, II, III represent sampling periods: July, August, and September. a, b = values within a sampling area with different letters are statistically significant ($p < 0,05$). * = statistically significant ($p < 0,05$) between the same periods of sampling different areas.

It is generally accepted that in ruminants, lipid mobilization leads to triglyceride accumulation in the liver and that the severity of triglyceride accumulation depends on the extent of lipomobilisation. Studies on fasted cows have suggested that increased hepatic uptake of free fatty acids associated with inadequate hepatic secretion of triglycerides may constitute a major mechanism for lipid accumulation in the liver (Mazur *et al.*, 2009). Such findings could be due to the effect of some factors such as age, sex, health status, and breed on serum lipids and lipoproteins profile. Rahman *et al.* (2018) conducted a study on an indigenous sheep breed in two different districts and found that although the levels of triglyceride and cholesterol should be related to each other, the levels of triglyceride were higher in young sheep. A possible cause for such findings could be a higher body fat percentage in young sheep. The same authors found no significant differences in cholesterol levels in different age groups. Moreover, geographic, seasonal, and dietary factors may affect the serum

concentrations of lipids and lipoproteins in sheep. It was reported that sheep under overgrazing conditions exhibited significantly reduced growth performance and changes in serum biochemical values in comparison to sheep under the light grazing conditions (Zhang *et al.*, 2019). Rathwa *et al.* (2017) found decreased levels of glucose and cholesterol during the summer season, in comparison to levels of the same parameters during the winter season, which could be due to decreased feed intake. The levels of cholesterol, triglycerides, and glucose can also be negatively affected by heavy metals (Kovacic *et al.*, 2017). Higher levels of cholesterol were found during the postpartum phase in Gaddi sheep, which can be due to increased steroidogenesis in the ovarium (Sharma *et al.*, 2015). The levels of cholesterol can also be influenced by breed. It was reported that Captive bighorn sheep, wild bighorn sheep, Awassi Sheep, and Coimbatore sheep have physiologically higher values of cholesterol levels in serum (Rahman *et al.*, 2018) acquired from supplemental food. Cholesterol is a source of energy and is a precursor of steroid hormones, bile acids, and also required for normal cell function.

CONCLUSION

Cholesterol and triglycerides are parameters that are indicators of the health of the body. Various internal and external factors can change the concentrations of cholesterol and triglycerides in serum, including age, sex, breed, season, geographical area, and diet type. A much higher sampling frequency is recommended to test the specificity of pramenka sheep breed and the variation of individual biochemical parameters.

REFERENCES

- Antunović, Z., Senčić, Đ., Šperanda, M., & Liker, B. (2002): Influence of the season and the reproductive status of ewes on blood parameters. *Small Ruminant Research*, 45(1), 39-44.
- Ašimović, Z. (2005): Izabrani parametri metabolizma kao indikatori specifičnosti dubskog soja pramenke. Doktorska disertacija. Poljoprivredno prehrambeni fakultet Sarajevo.
- Cerutti, R. D., Scaglione, M. C., Arfuso, F., Rizzo, M., & Piccione, G. (2018): Seasonal variations of some hematochemical parameters in Holstein bovine under the same livestock conditions. *Veterinarski arhiv*, 88(3), 309-321.
- Iriadam, M. (2007): Variation in certain hematological and biochemical parameters during the peri-partum period in Kilis does. *Small Ruminant Research*, 73(1-3), 54-57.
- Karapehliyan, M., Atakisi, E., Atakisi, O., Yucayurt, R., & Pancarci, S. M. (2007): Blood biochemical parameters during the lactation and dry period in Tuj ewes. *Small Ruminant Research*, 73(1-3), 267-271.

- Khan, A., Rehman, S., Imran, R., & Pitafi, K. D. (2013): Analysis of serum cholesterol level in goats breeds in Gilgit-Baltistan area of Pakistan. *Journal of Agricultural Science and Technology*, A, 3(4A), 302.
- Kiran, S., Bhutta, A. M., Khan, B. A., Durrani, S., Ali, M., & Iqbal, F. (2012): Effect of age and gender on some blood biochemical parameters of apparently healthy small ruminants from Southern Punjab in Pakistan. *Asian Pacific Journal of Tropical Biomedicine*, 2(4), 304-306.
- Kovacik, A., Arvay, J., Tusimova, E., Harangozo, L., Tvrda, E., Zbynovska, K., & Massanyi, P. (2017): Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters. *Chemosphere*, 168, 365-371.
- Mazur, A., Ozgo, M., & Rayssiguier, Y. (2009): Altered plasma triglyceride-rich lipoproteins and triglyceride secretion in feed-restricted pregnant ewes. *Veterinární medicína*, 54(9), 412-418.
- Mugabe, L. C., Bagaldo, A. R., Barbosa, L. P., Araújo, F. L. D., Oliveira, B. Y. D. S., Silva, R. V. M. M., & Pinheiro, E. E. G. (2017): Biochemical and seminal parameters of lambs fed palm kernel cake under grazing system. *Revista Brasileira de Zootecnia*, 46(8), 670-677.
- Nazifi, S., Saeb, M., & Abedi, M. (2003): Serum lipid profiles and their correlation with thyroid hormones in clinically healthy Turkoman horses. *Comparative Clinical Pathology*, 12(1), 49-52.
- Okonkwo, J. C., Omeje, I. S., Okonkwo, I. F., & Umeghalu, I. C. E. (2010): Effects of breed, sex and source within breed on the blood bilirubin, cholesterol and glucose concentrations of Nigerian goats. *Pak J Nutr*, 9(2), 120-124.
- Opara, M. N., Udevi, N., & Okoli, I. C. (2010): Haematological parameters and blood chemistry of apparently healthy West African Dwarf (Wad) goats in Owerri, South Eastern Nigeria. *New York Science Journal*, 3(8), 68-72.
- Rahman, M. K., Islam, S., Ferdous, J., Uddin, M. H., Hossain, M. B., Hassan, M. M., & Islam, A. (2018): Determination of hematological and serum biochemical reference values for indigenous sheep (*Ovis aries*) in Dhaka and Chittagong Districts of Bangladesh. *Veterinary world*, 11(8), 1089.
- Rathwa, S. D., Vasava, A. A., Pathan, M. M., Madhira, S. P., Patel, Y. G., & Pande, A. M. (2017): Effect of season on physiological, biochemical, hormonal, and oxidative stress parameters of indigenous sheep. *Veterinary world*, 10(6), 650.
- Schlumbohm, C., Sporleder, H. P., Gurtler, H., & Harmeyer, J. (1997): The influence of insulin on metabolism of glucose, free fatty acids and glycerol in normo- and hypocalcemic ewes during different reproductive states. *Deutsche Tierärztliche Wochenschrift*, 104(9), 359-365.
- Sharma, A., Kumar, P., Singh, M., & Vasishta, N. K. (2015): Haemato-biochemical and endocrine profiling of north western Himalayan Gaddi sheep during various physiological/reproductive phases. *Open veterinary journal*, 5(2), 103-107.

- Yokus, B., Cakir, D. U., Kanay, Z., Gulen, T., & Uysal, E. (2006): Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. *Journal of Veterinary Medicine Series A*, 53(6), 271-276.
- Zhang, J., Gao, Y., Guo, H., Ding, Y., & Ren, W. (2019): Comparative metabolome analysis of serum changes in sheep under overgrazing or light grazing conditions. *BMC Veterinary Research*, 15(1), 1-10.

ANALIZA SERUMSKOG HOLESTEROLA I TRIGLICERIDA U KRVI MLIJEČNIH OVACA ZAVISNO OD LOKALITETA UZORKOVANJA

Rezime

Cilj ovog istraživanja bio je praćenje koncentracija holesterola i triglicerida u serumu ovaca sa područja Livanjskog polja i planine Vlašić. U istraživanje je uključeno 117 ovaca pasmine pramenka. Ovce su podijeljene u grupe prema području uzorkovanja kroz različite periode (juli, august i septembar). Pomoću automatskog analizatora (Kodak Ektachem analizator DT 60 II, DTSC Modul i DTE Modul) u krvnom serumu ovaca vršeno je određivanje koncentracija biohemijskih parametara holesterola i triglicerida. Na području Livanjskog polja najveća ustanovljena vrijednost holesterola u serumu ovaca bila je u drugom periodu uzorkovanja, sa statističkom značajnošću u odnosu na druga dva perioda uzorkovanja. Vrijednosti holesterola na području planine Vlašić kretale su se u okviru fizioloških granica. Najveća ustanovljena koncentracija triglicerida na području Livanjskog polja bila je također u drugom periodu uzorkovanja, sa statističkom značajnošću ($p < 0,05$) u odnosu na ostale periode uzorkovanja, kao i na isti period sa područja Travnika. Dobiveni rezultati triglicerida u krvnom serumu ovaca sa područja planine Vlašić pokazuju tendencu snižavanja u trećem periodu uzorkovanja, odnosno na kraju perioda laktacije. Ovi rezultati mogu biti posljedica djelovanja različitih faktora, poput dobi, spola, zdravstvenog stanja, statusa laktacije, ali i pasmine ovaca.

Keywords: *ovca, serum, holesterol, trigliceridi*