Bulgarian Journal of Agricultural Science, 25 (Suppl. 1) 2019
Agricultural Academy

Haematological and blood biochemical parameters in Lacaune dairy sheep

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Abstract

Nedeva, I., Slavov, T., Varlyakov, I., Radev, V., & Panayotov, D. (2019). Haematological and blood biochemical parameters in Lacaune dairy sheep. *Bulgarian Journal of Agricultural Science, 25*(Supp 1), 91–95

The effect of productivity (high and low) and season (winter vs summer) on venous blood morphology and biochemical parameters was investigated in dairy Lacaune sheep. Thirty-two animals were included in the experiment, divided into 2 groups. It was found out that during the winter, white blood cell counts, red blood cell counts, thrombocytes, haemoglobin content, haematocrit and mean corpuscular haemoglobin increased both in high-productive and low-productive sheep ($p < 0.05 – p < 0.001$). Blood liver enzymes (ASAT, gamma glutamyl transferase, alkaline phosphatase) exhibited elevation during the winter ($p < 0.05 – p < 0.01$). No seasonal influence has been observed with respect to the other assayed haematological (MCH, MCV, MCHC, RDW, MPV, PDW, PCT) and blood biochemical (glucose, creatinine, total cholesterol, triglycerides, Ca, P, total protein, albumins, globulins) parameters.
Keywords: Breed Lacaune; blood sample; morphology; biochemistry; indicators

Introduction

In 1989 Bulgaria has moved from a centrally planned to a market-oriented economy, accompanied with a decrease in sheep population from 10 to 1.3 million which posed a risk for disappearance of some breeds (Tyankov et al., 2000). Sabkov et al. (2017) affirmed that the country possesses all the prerequisites for the development of intensive milk sheep breeding due to the location, climatic conditions, the rich forage base and last but not least, the traditions and experience in sheep farming.

Sheep milk production occupies a significant part of agriculture in many countries. The largest global sheep milk producer is China with 12.2%. The leading producer of sheep milk in Europe is Greece (8.7%) followed by Romania (7.2%) and Italy (6.1%, Barlowska et al., 2011). Because of its high nutritional value due to significant concentrations of proteins, fats, vitamins and minerals as compared to milk from other domestic animals, sheep milk is a delicacy product in many countries, including the USA (Park et al., 2007; Milani & Wendorff, 2011). Sheep milk is usually used for production of fine cheeses, yogurt and whey cheese. Lacaune is among the most high-yielding dairy sheep breeds, specially selected to meet the needs from sheep milk of dairy industry in Roquefort region, where the world-famous blue cheese is produced. According to Berger (2004), the Lacaune breed is crossed with other breeds to improve the milk fat quality. The production of high-quality sheep milk as a natural source of anticarcinogenic substances is depending on the sward composition, ecological features of the region and breed's features. Milk fat provides essential fatty acids to the body. Sheep milk is a primary source of conjugated linoleic acid (CLA) whose amount varies depending on the breed, season and feeding regimen (Angelov et al., 2012; Odzhakova et al., 2012).

The analysis of blood parameters is an important method for evaluation of nutritional and health status of livestock. According to Hristev (2007) up to 70-80% of animal productivity could he attribute to feeding, season and rearing conditions? A more detailed study could outline the effect of various feeding systems (Yurtman et al., 1997). The effect of different biological and technological factors on systemic immune status was studied, but the role of genetic factors (including the breed) and some others as age, productivity were less investigated.

The aim of the study was to investigate the effect of productivity (high and low) and season (winter vs summer) on venous blood haematological and biochemical parameters in lactating dairy Lacaune sheep.

Materials and Methods

The study was carried out in the elite farm for Lacaune sheep in the Saedinenie region, Plovdiv district. At present, 2600 ewes are housed in the farm, which produce milk all year round. The sheep are reared in stalls under controlled microclimatic conditions. The sheep farm has a modern Parallel 2x36 milking parlour with rapid exit, equipped with software for herd management DeLaval DelPro. Thirty two dairy sheep were analyzed during two different seasons – winter and summer. They were divided into 2 groups according to their milk yield on the basis of data from the herd management software for the previous lactation. Low-productive sheep were those with minimum yield < 1.5 L, and high-productive: with minimum yield >2.2 L. During the summer, sheep were fed a ratio with alfalfa haylage 2 kg, alfalfa hay 0.8 kg, meadow hay 0.5 kg, alfalfa pellets 0.6 kg, barley 0.4 kg, sugar beet pellets 0.225 kg. The total amount of daily ratio for a sheep was 5.44 kg, providing 2.93 milk feeding units and dietary crude protein level of 16%. The winter diet comprised alfalfa hay 0.6 kg, meadow hay 0.5 kg, barley straw 0.6 kg and barley 0.5 kg. The total amount of daily ration for a sheep was 2.2 kg, providing 1.43 milk feeding units and dietary crude protein level of 10%. The feeding at the farm was performed with total mix ration and feed dispenser and mixer.

Blood samples were collected from v. jugularis externa from a licensed veterinarian. Samples were transported in a cooled bag to the certified lab of the National Centre for Professional Training and Competence America for Bulgaria to the Trakia University – Stara Zagora. Blood was assayed on automated haematological analyser BC-2800VET and automated biochemical analyser BS-120. Assayed CBC parameters were: white and red blood cell counts, haemoglobin, haematocrit, thrombocytes etc. Blood serum parameters were blood glucose, creatinine, urea, ASAT, ALAT, GGT, alkaline phosphatase etc.

Results and Discussion

Table 1 presents the results from blood morphology in dairy Lacaune sheep. Leukocyte counts (WBC) in high-productive animals increased statistically significant ($p < 0.01$) up to 15.6 x10^9/L during the winter. The same was observed in low-productive sheep during the same season – statistically significant increase up to 19.53 x10^9/L ($p < 0.001$). Leukocytes play an important role in systemic protection and repair in animals. Blood leukocyte counts depend on the genotype, breed, age, physiological condition etc. The higher WBC counts in both groups of sheep in the winter are attributed to the enhanced metabolism, the maintenance of constant body temperature and physiological condition.

Blood parameters change in animals exposed to heat stress. Gomes de Silva (1992) established reduced WBC counts in animals under heat stress. Others reported higher WBC counts in animals in conditions of high ambient temperatures (Wojtas, 2014). Other studies of ours with Lacaune sheep breed (Slavov et al., 2018) demonstrated increased total leukocyte counts due to the production of more milk during the second lactation. WBC in animals changes under the influence of the central nervous system as induced by unconditional and conditional refectory changes. Data for the breed-, season- and milk yield-related changes of sheep blood leukocytes are few or lacking (Boychev, 2012). Leukocytosis, resp. leukopaenia could be due to inflammations, enhanced metabolic rate during the pregnancy, under the influence of toxic substances, stress, infectious diseases, starvation etc. (Fishman & Hofman, 2004; Harris, 2006). The analysis showed that erythrocytes in high-yielding sheep were 9.01 x10^{12}/L in the summer, and in the winter increased insignificantly to 9.22 x10^{12}/L. In low-yielding sheep, summer RBC counts were 8.5 x10^{12}/L, and in the winter, they increased statistically significantly ($p < 0.01$) to 9.87 x10^{12}/L (Table 1). Lower erythrocyte counts in Lacaune sheep during the summer could result from the lower nutrition level and the reduced appetite consequently to the hot season. Previous studies have evidenced that lower feed intake is due to increased ambient temperatures in summer (Silankove et al., 1980; More et al., 1981, Abdelatif et al., 2009).

Lower RBC counts in sheep could be partly due to haemodilution. Haemoglobin content in low-productive sheep was considerably higher in the winter vs summer values, while in high-productive sheep, it did not change a lot with the season (Table 1). Haematocrit was directly dependent on increased RBC and haemoglobin in low-productive

Table 1. Blood morphological parameters in high-productive and low-productive Lacaune sheep during the summer and winter

Parameter	High-productive (n = 17)				Low-productive (n = 15)				Significance High/Low
	Summer		Winter		Summer		Winter		
	x	±Sx	x	±Sx	x	±Sx	x	±Sx	
WBC 109/l	9.48	0.79	15.6	1.37	7.84	0.48	19.53	0.76	**/***
RBC 1012/l	9.01	0.48	9.22	0.34	8.5	0.22	9.87	0.32	NS/**
HGB (g/l)	99.41	2.89	108.94	4.07	85.73	2.09	114.33	4.07	NS/***
HCT (%)	30.35	1.61	32.39	1.14	28.47	0.65	32.49	1.38	NS/*
PLT (109/l)	251.81	11.61	207.88	12.51	269.53	20.05	217.2	16.48	*/*

Statistically significant differences between summer and winter in in high-productive and low-productive sheep: *- ($p < 0.05$); **- ($p < 0.01$); ***- ($p < 0.001$); NS – not significant

sheep during the winter and increased to 32.49 % ($p < 0.05$). Blood haemoglobin content is directly associated with red blood cells. Lower haemoglobin during the summer is due to lower feed intake during the hot season (Abdelatif et al., 2009). Blood thrombocyte counts in high-yielding Lacaune sheep increased to 251.81 x10^9/L in the summer ($p < 0.05$), while they were reduced in the winter. In low-productive sheep, thrombocytes increased to 269.53 x10^9/L ($p < 0.05$) in the summer vs winter counts (217.2 x10^9/L). Slavov et al. (2018) found out the thrombocyte counts in Lacaune sheep increased during the second lactation due to enhanced milk production.

Blood biochemical parameters in high- and low-productive sheep are presented in Table 2. In the former, summer blood glucose was 2.86 mmol/L, which increased statistically insignificantly to 2.97 mmol/L in the winter. Similar results were observed in low-productive sheep, in which summer glucose was 2.83 mmol/L vs winter value of 3.12 mmol/L. When an animal is exposed to different stressors, blood cortisol concentrations increase consequently to stimulation of hypothalamo-pituitary-adrenal axis. Increase cortisol could lead to higher blood glucose levels. Apart that, the enhanced sympathetic adrenal activity triggered by physiological and psychic stress, could also lead to hyperglycaemia via enhanced liver glycogen degradation (Adenkola & Ayo, 2010; Ali et al., 2006). Despite the increase, the parameter was within the reference range. Other studies on blood parameters in Lacaune sheep (Slavov et al. 2018) reported increased blood glucose during the first lactation as compared to the second one, due to increased milk yields and release of lactose in milk. Antunović (2002) reported a seasonal influence on lowered blood sugar. Blood creatinine in both groups varied between 61 and 64.24 µmol/L in both seasons (Table 2). During the summer blood urea in high-yielding sheep was 11.52 mmol/L, and in winter it decreased ($p < 0.001$) to 7.34 mmol/L. Comparable tendency was noted for low-productive sheep – increase in the summer to 10.93 mmol/L and reduction in the winter to 6.78 mmol/L ($p < 0.001$). Blood bilirubin increased in high-productive sheep to summer values of 37.08 µmol/L ($p < 0.05$) as compared to winter: 26.55 µmol/L. There was a minor decrease in blood bilirubin in low-productive sheep to 30.96 µmol/L in the winter season (Table 2).

Nitrogen compounds urea and creatinine are among the end products of protein metabolism. Blood creatinine in animals indicated the function of kidneys and high concentrations are marker of impaired renal function. In this study season was found to have no influence on creatinine. Varlyakov et al. (2015) reported slight increase in blood creatinine in yearling rams after supplementation of their diet with Opti-

Table 2. Blood biochemical parameters in high-productive and low-productive Lacaune sheep during the summer and winter

Parameter	High-productive (n = 17)				Low-productive (n = 15)				Significance High/Low
	Summer		Winter		Summer		Winter		
	x	±Sx	x	±Sx	x	±Sx	x	±Sx	
GLU mmol/l	2.86	0.07	2.97	0.1	2.83	0.13	3.12	0.15	NS/NS
CREA µmol\l	64	2.06	64.24	1.59	61	2.31	62.13	1.88	NS/NS
UREA mmol/l	11.52	0.44	7.34	0.28	10.93	0.41	6.78	0.29	***/***
Tbili µmol\l	37.08	4.26	26.55	2.11	35.94	3.63	30.96	3.69	*/NS

Statistically significant differences between summer and winter in in high-productive and low-productive sheep: *- ($p < 0.05$); **- ($p < 0.01$); ***- ($p < 0.001$); NS – not significant gen both before and after feeding. Slavov et al. (2014) found out higher blood urea levels in yearling rams whose diet was supplemented with Zarnela. The addition of Optigen to the diet of yearling rams resulted in statistically significant increase of blood urea 2.5 hours after feeding (Varlyakov et al., 2015). Having tested various rations, Sivkova (2007) demonstrated the highest blood urea concentrations in diets with sunflower meal both before and 2.5 h after feeding. Osborne et al. (2002) reported reduced blood urea after dietary supplementation with sugars, while others did not (Sannes et al., 2002; Ordway et al., 2002). Mc Cormik et al. (2001) observed increase in blood glucose after supplementation of the ratio with sugars.

Summer blood ASAT activity in high-producing sheep was 39.35 U/l and increased statistically significantly ($p < 0.001$) to 102.65 U/l. In low-productive animals, summer ASAT was 55.47 U/l and then increased to 106.07 U/l ($p < 0.01$; Table 3). Alkaline phosphatase in blood of high-yielding Lacaune sheep was 98.94 U/l in the summer and as substantially elevated in the winter (171.53 U/l; $p < 0.01$). The summer AP activity in low-yielding sheep was 102.93 U/l and increased to 178.67 U/l ($p < 0.05$) GGT activity in the summer was 28.67 U/l in low-productive animals while in the winter – 57.87 U/l ($p < 0.05$). In high-producing sheep, blood GGT was 46.18 U/l in the summer and increased slightly to 51.59 U/l (Table 3). The numerous cellular enzymes have a clinical relevance as well. In the liver, L-aspartate oxoglutarate aminotransferase (ASAT), L-alanine 2-aminotransferase (ALAT), gamma-glutamyltransferase (GGT) and alkaline phosphatase (AP) exhibit high activity and most frequently are assayed to confirm acute or chronic liver diseases (Steen, 2001). In a previous study of ours in Lacaune sheep reared on pastures, a similar increase in blood liver enzymes was noted (Slavov et al., 2018).

The performed haematological analyses in Lacaune sheep reared in stables all year round, we confirmed a seasonal influence on blood parameters in high- and low-yielding animals. Statistically significant increase in leukocytes, erythrocytes, haemoglobin and haematocrit occurred in both groups in the winter, while in thrombocytes – in the summer. From blood biochemical indices, statistically significant increase was established in blood urea and bilirubin in both groups during the summer and higher activities of liver enzymes (ASAT, ALAT, GGT, AP) during the winter. All other studied parameters (MCV, MCHC, RDW, MPV, PDW, PCT, glucose, creatinine, total cholesterol, triglycerides, Ca, P, total protein, albumin, globulins) did not change substantially. Our results are in line with those reported in sheep (Gupta et al. 2008).

Conclusions

The present study allowed concluding that:

✓ During the winter, white blood cell counts increased both in high-productive ($p < 0.01$) and low-productive sheep ($p < 0.001$), possibly due to enhanced metabolism

✓ The winter season was also accompanied with increased red blood cell counts ($p < 0.01$), haemoglobin content ($p < 0.001$), haematocrit ($p < 0.05$) and mean corpuscular haemoglobin ($p < 0.001$) in low-productive sheep.

✓ Winter activities of ASAT and alkaline phosphatase in the blood of high-yielding Lacaune sheep were elevated ($p < 0.01$-$p < 0.001$).

✓ In low-productive sheep, gamma-glutamyltransferase and ASAT in blood were increased in the winter ($p < 0.05$-$p < 0.01$).

✓ The thrombocyte counts in both groups of sheep was higher during the summer as compared to the winter ($p < 0.05$).

References

Abdelatif, A. M., Ibrahim, M. Y. & Hassan, Y. Y. (2009). Seasonal variation in erythrocytic and leukocytic indices and serum proteins of female Nubian Goats. *Middle-East Journal of Scientific Research*, *4*, 168-174.

Adenkola, A. Y. & Ayo, J. O. (2010). Physiological and behavioral responses of livestock to road transportation stress: A review. *African Journal of Biotechnology*, *9*, 4845-4856.

Table 3. Blood serum enzymes in high-productive and low-productive Lacaune sheep during the summer and winter

Parameter	High-productive (n = 17)				Low-productive (n = 15)				Significance High/Low
	Summer		Winter		Summer		Winter		
	x	±Sx	x	±Sx	x	±Sx	x	±Sx	
ASAT U/I	39.35	3.95	102.65	11.69	55.47	4.17	106.07	12.37	***/**
ALAT U/I	17.71	0.64	16.53	1.14	17.67	0.86	15.73	1.12	NS/NS
GGT U/I	46.18	3.15	51.59	4.11	28.67	3.26	57.87	9.45	NS/*
ALP U/I	98.94	14.63	171.53	16.84	102.93	20.14	178.67	31.08	**/NS

Statistically significant differences between summer and winter in in high-productive and low-productive sheep * – ($p < 0.05$); ** – ($p < 0.01$); *** – ($p < 0.001$); NS – not significant

Ali, B. H., Al-Qarawi, A. A. & Mousa, H. M. (2006). Stress associated with road transportation in desert sheep and goats, and the effect of pre-treatment with xylazine or sodium betaine. *Research in Veterinary Science*, 80, 343-348.

Angelov, L., Ivanova, S., Odzhakova, Ts. & Gadzhev, D. (2012). Changes in milk fatty acid profile and the content of biologically active substances with anticarcinogenic effect in Rhodope Tsigay sheep reared in Middle Rhodope Mountain. Natural sciences, mathematics and informatics (Natural and agrarian sciences. Medicine), II, part I, 291-298, Plovdiv University "Paisii Hilendarski", Smolyan (Bg).

Antunović, Z., Senčić, D., Šperanda, M. & Liker, B. (2002). Influence of the season and the reproductive status of ewes on blood parameters. *Small Ruminant Research*, *45***,** 39–44.

Barłowska, J., Szwajkowska, M. Litwinczuk, Z. & Kr´ol, J. (2011). Nutritional value and technological suitability of milk from various animal species used for dairy production. *Comprehensive Reviews in Food Science and Food Safety*, *10*, 291–302.

Berger, Y. M., (2004). Breeds of sheep for commercial milk production. *Spooner Agricultural Research Station*, University of Wisconsin-Madison, Madison, Wisconsin, USA.

Boychev, K, (2012). Breed-related changes in white blood cells in sheep blood depending on the season. *Animal Science (Sofia)*, XLIX(4), 17-24 (Bg).

Fishman, M. & Hofman, A. (2004). Medicine. Lippincott Williams and Wilkins, New York.

Gomes da Silva, R., Paranhos da Costa, M. J. R. & Silva Sobrinho, A. G. (1992). Influence of hot environments on some blood variables of sheep. *International Journal of Biometeorology*, *36*, 223–225.

Gupta, V. K., Sharma, S. D., Vihan, V. S. & Kumar, A. (2008). Prevalence and changes in haemogram in subclinical ketosis in sheep reared under organized farming system. *The Indian Journal of Animal Sciences*, *78*, 453-456.

Harris, J., (2006). Blood Cell Biochemistry. Springer, Berlin, New York.

Hristev, H, (2007). Innate Resistance in Animals, Agricultural University, Plovdiv, 128.

Mc Cormik, M. E., Redfearn, D. D., Ward, J. D. & Bloun, D. C. (2001). Effect of protein source and soluble carbochydrate addition on rumen fermentation and lacting performance of Holstein cows. *Journal of Dairy Science*, *84*, 1686-1697.

Milani, F. X. & Wendorff, W. L. (2011). Goat and sheep production in the United States (USA). *Small Ruminant Research*, *101*, 134-9.

More, T. & Sahni, K. L. (1981). Effect of water intake on feed digestibility. *World Review of Animal Production*, *17*, 33-39.

Odzhakova, Ts., Ivanova, S., Gadzhev, D. & Angelov, L. (2012). Effect of geochemical features of high-mountain pastures in Middle Rhodope Mountain on milk fatty acid and of biologically active substances with anticarcinogenic effect content in Karakachan sheep. Natural sciences, mathematics and informatics (Natural and agrarian sciences. Medicine), II, part I, 281-290, Plovdiv University "Paisii Hilendarski", Smolyan (Bg).

Ordway, R. S., Ishler, V. A. & Varga, G. A. (2002). Effects of sucrose supplementation on dairy matter intake, milk yield, and blood metabolites of periparturient Holstein dairy cows. *Journal of Dairy Science*, *85*, 879-888.

Osborne, V. R., Leslie, K. E & McBride, B. W. (2002). Effect of supplementing glucose in drinking water on energy and nitrogen statud of the transition dairy cows. *Canadian Journal of Animal Science*, *82*, 427-433.

Park, Y.W., Júarez, M., Ramos, M. & Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminants Research*, *68*, 88–113.

Sabkov, H., Stoycheva, S. & Ivanova, T. (2017). Trends in the development of sheepbreeding and approach for introduction of machine milking of sheep in R. Bulgaria. *Journal of Mountain Agriculture on the Balkans*, *20*, 1-17.

Sannes, R. A., Messman, M. A. & Vagnoni, D. B. (2002). Form of rumen-degradable carbohydrate and nitrogen on microbial protein synthesis and protein efficiency of dairy cows. *Journal of Animal Science*, *85*, 900-908.

Silankove, N. H., Tagari, H. & Shkolnik, A. (1980). Gross energy digestion and urea recycling in the desert Black Bedouin goat. *Comparative Biochemistry and Physiology*, *67*, 215-218.

Sivkova, K., Enev, E., Radev, V., Nedeva, N. & Slavov, T. (2007). Effect of diet structure on some parameters of carbohydrate metabolism in the rumen, duodenum and blood in sheep. *Animal Science*, *6*, 61-68 (Bg).

Slavov, T., Panayotov, D., Nedeva, I., Radev, V. & Varlyakov, I. (2018). Haematological parameters in lacaune ewes associated to the parity. *Bulgarian Journal of Agricultural Science*, *24*, 82-87.

Slavov, T., **Radev, V. & Varlyakov, I.** (2014). Effect of dietary supplementation with dried distillers grain with solubles (Zarnela) on blood parameters in yearling sheep. *Journal of animal science*, *LI*, 80-86 (Bg).

Steen, A., (2001). Field study of dairy cows with reduced appetite in early lactation: clinical examinations, blood and rumen fluid analyses. *Acta Veterinaria Scandinavica*, *42*, 219-228.

Tyankov, S., Dimitrov, I., Stankov, I., Slavov, R. & Panayotov, D. (2000). Sheep breeding and Goat breeding. Abagar, Veliko Tarnovo, pp. 588 (Bg).

Varlyakov, I., Radev, V., Slavov, T., Uzunova, K., Mihaylov, R. & Toshevska, M. (2015). Changes in Blood BiochemicalIndices in Yerling Rams after Dietary Supplementation of Optigen. *Albanian Journal of Agricultural Science*, *14*, 19-24

Wojtas, K., Cwynar, P. & Kołacz, R. (2014). Effect of thermal stress on physiological and blood parameters in merino sheep. *Bulletin of the Veterinary Institute in Pulawy*, *58*, 283-288.

Yurtman, I. Y., Polatsu, S., Baspinar, E. & Ozduven, L. M. (1997). The effects of different raw protein levels on some blood metabolites in sheep in lambs. (Farkili Ham Protein Düzeylerinin Yogun Besideki Türkgrldi Kuzularinda Bazi Kan Metabolitlerine Etkileri). *Veteriner Bilimler Dergi*, *13*, 17-24.