Bulgarian Journal of Agricultural Science, 24 (Supplement 1) 2018, 82-87

HAEMATOLOGICAL PARAMETERS IN LACAUNE EWES ASSOCIATED TO THE PARITY

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Abstract

SLAVOV, T., D. PANAYOTOV, I. NEDEVA, V. RADEV and I. VARLYAKOV, 2018. Haematological parameters in Lacaune ewes associated to the parity. *Bulg. J. Agric. Sci.*, 24 (Suppl. 1): 82–87

The aim of the present study was to evaluate the changes in blood haematological parameters in Lacaune ewes at the time of the first and second lactation. To achieve the aim 26 ewes divided in two groups (first and second lactation) were investigated. Higher white blood cell counts ($p<0.001$), platelets and plateletcrit ($p<0.05 – p<0.01$) were established in the ewes in second lactation. It was also demonstrated that haemoglobin and haematocrit were similarly elevated in the second-lactation ewes ($p<0.05 – p<0.01$). Blood total protein, albumin and globulins in Lacaune ewes varied within the normal physiological range during the first and second lactation.

Key words: sheep, Lacaune breed, lactation, blood parameters

Introduction

Lacaune is one of the highest yielding dairy sheep breeds in the world. It is reared in southern France in the departments Tarn, Hérault, and Aveyron. It is created in the 19th century on the basis of several local sheep populations. In the early period of its creation, blood from half-merino and Southdown meat breed was introduced. The creation of the Lacaune breed was necessitated by the need to supply a sufficient amount of sheep milk for dairy industry in the Roquefort region, producing the world-famous Roquefort cheese. Lacaune population is the most numerous in France, also the most important and efficient comprising more than 1 300 000 animals e.g. about 20% of all sheep reared in France; and 840 000 out of them are breeders. They are well acclimated to mountainous regions with altitude 300-1000 m and dry climate. The ewes weigh 70-80 kg, and rams - 95-120 kg. Prolificacy is about 190-200%. The average milk yield per lactation is 350-370 L. In France, sheep from this breed in selection flocks produce 280 L milk per 165-day lactation (Barillet et al., 2001). According to Berger (2004) the Lacaune breed is used for crossing with other breeds in order to improve the quality of milk fat. The production of high-quality sheep milk as a natural source of anticarcinogenic substances and biologically active substances is determined by pastures' sward, ecological features of the region and individual traits of the breed. Milk fat provides the essential fatty acids to the body. Sheep milk is a main source of conjugated linoleic acid (CLA) whose amount varies according to 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 the nutritional and health status of livestock. According to Hristev (2007) up to 70-80% of animal productivity is determined by nutrition, season and rearing conditions. A more detailed analysis could indicate the effects of various feeding regimens (Yurtman et al., 1997). Various biological and technological factors influencing immune status of the body have been studied, but the role and importance

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of genetic factors (breed, age, and productivity) are less thoroughly examined.

In Bulgaria, haematological parameters in Lacaune sheep are not sufficiently investigated. The changes in blood parameters associated to the number of lactation are also not monitored. This was the main purpose of the present research.

Material and methods

A total of 26 Lacaune ewes were included in the study. The animals were reared in a private farm owned by Yanko Mitev located in Vodenichane village, Yambol district. The first sheep from the breed were imported at the farm from France in 2007. At present, the number of sheep subject to selection control is 200. The ewes were divided into 2 groups: at first and second lactation, comprising 13 animals each. During the first lactation lasting 150 days, daily milk yield was 1.8 L, and during the second lactation - 2.5 L. Ewes were reared mainly on pasture, and supplemented with 1 kg concentrate containing 18% crude protein, manufactured by HL-TopMix – Sliven. Ewes were reared indoor, with constant access to drinking water and salt licks.

Blood samples were collected immediately after milking from *v. jugularis externa* in vacutainers without anticoagulant for isolation of blood serum and in vacutainers containing EDTA.K2 for complete blood count determination. Samples were transported in a cooling bag to the certified laboratory of the National Centre for Professional Training and Competence "America for Bulgaria" at the Trakia University – Stara Zagora and Bodylab – Sliven. Blood was assayed on automated haematological analyzer BC-2800VET and automated biochemical analyzer BS-120. Anticoagulated blood was analysed for CBC comprising: white blood cells (WBC); red blood cells (RBC); haemoglobin (HGB); haematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); red blood cell distribution width (RDW); platelets (PLT); mean platelet volume (MPV); platelet distribution width (PDW); plateletcrit (PCT). The following parameters were assayed in blood serum: blood glucose; creatinine; urea; total cholesterol triglycerides; aspartate aminotransferase; alanine aminotransferase; gamma glutamyltransferase; alkaline phosphatase; total protein; albumin; globulins.

Results and discussion

White blood cell counts (Table 1) in Lacaune sheep was $12.11x10^9$/L during the first lactation and increased up to $15.95x10^9$/L in ewes at second lactation. The difference was statistically significant ($p<0.001$). The increased need from plasma proteins for production of more milk during the second lactation has resulted in increased total leukocyte counts. Along with characteristic normal variations in the parameters with respect to metabolic reactions and body physiology, increased white blood cell counts (leukocytosis) or their decrease (leukopaenia) vs reference ranges could result from inflammations, intoxications, irradiation, stress etc. or during infectious diseases, starvation, suppression of bone marrow functions etc. (Fishman and Hofman, 2004; Harris, 2006). Especially in sheep, apart the heredity effects on this CBC parameter (Semerdjiev, 1999; Mostaghni et al., 2005; Tibbo et al., 2005), the information on the integral effects of biotic and abiotic factors and seasonal influences on these parameters is scarce (Petrova et al., 1990; Mot et al., 2011).

Red blood cells are a primary part of blood content. Their counts depend on feeding, climate, physiological conditions, productivity, etc. Erythrocytes in sheep range within a broad reference interval – from $5x10^{12}$/L to $9x10^{12}$/L (Petkov et al., 2000). In our studies, RBC in sheep in first lactation were $7.07x10^{12}$/L, while during the second lactation when milk yield was higher – RBC increased up to $7.63x10^{12}$/L (Table 1). In high-yielding animals, blood RBC counts are higher (Petkov et al., 2000).

Apart being a primary constituent of red blood cells and responsible for the specific red colour, haemoglobin performs the respiratory function of blood. From chemical point of view it is a haemoproteid with protein component globin and a non-protein active prosthetic group, containing iron. Blood haemoglobin in Lacaune ewes is presented in Table 1. In ewes in first lactation it was 78.77 g/L, and during the second lactation – 85.77 g/L ($p<0.05$). The increase in the ewes in second lactation was directly associated with increased haemoglobin content and haematocrit, which increased up to 25.44 % ($p<0.01$).

Platelets perform a defense function, mainly in blood clotting. Reference values reported in the literature are from 170 to $980x10^9$/L, e.g. a very broad range. In our study, platelet counts in primiparous ewes were $212.23x10^9$/L, while during the second lactation along with increased milk production they increased with statistical significance ($p<0.05$) to $335.77x10^9$/L (Table 1). Plateletcrit (PCT) also increased in second-lactation ewes ($p<0.01$) up to 0.19% compared to first-lactation ewes (0.12%).

Blood glucose concentration (Table 2) in primiparous ewes was 4.11 mmol/L at average, while during the second lactation it decreased slightly to 3.84 mmol/L due to increased milk yield and release of lactose with milk. Regardless of the decrease, blood glucose was within the reference range. It is acknowledged that in ruminants, more than 30% of glucose in the liver is synthesised from amino acids (Bergman, 1973),

Table 1
Blood morphological parameters in Lacaune ewes during the first and second lactation

Lactation	Blood morphological parameters				
	n	x	$\pm S_x$	x	±Sx
	WBC (10^9/L)			MCMC (g/L)	
First lactation	13	12.11	2.59	339.69	4.34
Second lactation	13	15.95***	6.29	336.54	3.2
	RBC (10^{12}/L)			RDW (%)	
First lactation	13	7.07	0.18	14.83	0.17
Second lactation	13	7.63	0.23	14.75	0.16
	HGB (g/L)			PLT (10^9/L)	
First lactation	13	78.77	1.94	212.23	21.68
Second lactation	13	85.77*	1.79	335.77*	36.77
	HCT (%)			MPV (fl)	
First lactation	13	23.15	0.49	5.62	0.05
Second lactation	13	25.44	0.46	5.6	0.07
	MCV (fl)			PDW	
First lactation	13	32.92	0.51	14.77	0.07
Second lactation	13	33.59	0.51	14.55	0.06
	MCH (pg)			PCT (%)	
First lactation	13	11.12	0.18	0.12	0.01
Second lactation	13	11.25	0.21	0.19**	0.02

Statistically differences between Ist and IInd lactation: *- P<0.05; **-P<0.01; ***- P<0.001.

Table 2
Blood biochemical parameters in Lacaune ewes during the first and second lactation

Lactation	Blood biochemical parameters				
	n	x	$\pm S_x$	x	±Sx
	Blood glucose (mmol/L)			Triglycerides (mmol/L)	
First lactation	13	4.11	0.12	0.16	0.01
Second lactation	13	3.84	0.09	0.16	0.02
	Creatinine (μmol/L)			Total protein (g/L)	
First lactation	13	57.54	2.09	77.1	1.86
Second lactation	13	56.54	1.05	78.02	2.79
	Urea (mmol/L)			Albumins (g/L)	
First lactation	13	8.65	0.36	33.62	0.42
Second lactation	13	8.37	0.27	34.4	0.53
	Total cholesterol (mmol/L)			Globulin (g/L)	
First lactation	13	1.2	0.04	43.48	1.61
Second lactation	13	1.35	0.08	43.62	3

maximum 10% from glycerol (Bergman, 1971) and 36-76% - from propionic acid (Seal and Reynolds, 1993). A seasonal effect manifested with blood sugar reduction in sheep was reported (Antunovic´ et al., 2002).

From organic blood constituents, plasma proteins are the most important. Our results showed that serum total protein, regardless of the number of lactation, ranged within a narrow range from 77.1 g/L to 78.02 g/L. During the second lactation, a slight increase was noted. Sivkova et al. (2007) has established that as dietary beet content in sheep ration increased, blood total protein decreased both before and after feeding. Similar data were reported for blood total protein in lambs and yearling sheep by Grigorova et al. (2009), Todorova et al. (2009), Grigorova et al. (2010) who did not found out statistically significant changes after feeding various rations. After supplementation of sheep diets with enzyme preparations Hostazym C100 and Hostazym X100, blood total protein tended to increase. The supplementation of coconut oil to three different rations resulted in substantial increase in these parameters as affirmed by Slavov (2013).

Globulin concentration ranged from 43.48 – 43.62 g/L and followed the tendencies described for total protein (Table 2). Globulins are divided into alpha, beta and gamma fractions. They have a crucial role in immunity and systemic defense. Our results are within the reference values for sheep (35-55 g/L). They are also similar to values reported in previous studies of ours and by other researchers (Tosev, 1975; Varlyakov and Radev, 1998; Sivkova et al., 2007; Radev et al., 2011; Slavov, 2013).

Albumins are a main source for protein synthesis in organs. Their blood levels in first- and second-lactation sheep were 33.62 g/L and 34.40 g/L respectively, within the reference range for sheep (30-37 g/L). In our previous studies we have demonstrated that albumin in the blood of yearling sheep was influenced by dietary supplementation with enzyme preparations, and according to Caldeira et al. (2007) albumins and urea were the best indices for the levels of protein metabolism in ruminants.

Urea and creatinine are among the end products of protein metabolism. Blood creatinine indicates the normal functioning of kidneys, and high concentrations suggest that the renal function is impaired. In our studies, blood creatinine (Table 2) was from 56.54 to 57.54 μmol/L, therefore the number of lactation did not influence blood creatinine concentrations. Varlyakov et al. (2015) established a slight statistically insignificant increase in creatinine levels in blood after supplementation of Optigen to the ration of yearling sheep both prior to and after feeding.

The results for blood urea concentrations (Table 2) indicated that they varied within a very narrow range (8.37-8.65 mmol/L). Slavov et al. (2014) observed a statistically significant increase in blood urea ($P<0.001$) in yearling rams fed Zarnela. In other studies of our team (Varlyakov et al., 2015) an obvious effect of Optigen supplementation to the ratio of yearling sheep on blood urea was noted – it increased with statistical significance 2.5 hours after feeding ($p<0.05$). Having tested various rations, Sivkova (2007) demonstrated the highest urea concentrations in blood with rations containing sunflower meal. Decreased plasma urea was found out after addition of sugars to the diet by Osborne et al. (2002), while Sannes et al. (2002) and Ordway et al. (2002) did not observe such effect. Others (McCormik et al., 2001) reported increased blood plasma urea after feeding sugars with the ration.

Lipids are a group of natural organic compounds with different structure but similar physicochemical properties - triglycerides, free and esterified cholesterol, phospholipids (phosphatidylcholine, sphingomyelin, lysophosphatidylcholine and phosphatidylethanolamine), glycolipids, fat-soluble vitamins (A, D, E, K), carotenoids and bile acids. Total cholesterol and triglyceride concentrations are very informative. The results from our study with respect to total cholesterol and triglycerides in the blood of Lacaune ewes (Table 2) showed total cholesterol of 1.20 mmol/L (first lactation) and slight increase to 1.35 mmol/L during the second lactation. Slavov (2013) established that after dietary supplementation with coconut oil, blood total cholesterol and HDl cholesterol increased 2.5 h after feeding ($p<0.001$).

Blood serum triglycerides remained the same in sheep in first or second lactation (0.16 mmol/L). Slavov (2013) reported unchanged blood triglycerides after feeding ration I and II regardless of the additional amount of lipids introduced in the rumen of yearling sheep. After feeding ration III, the parameter increased slightly before feeding and tangibly – 2.5 h after feeding.

Cellular enzymes are numerous and of clinical relevance. They occur in the circulation after disruption of cellular membrane permeability or destruction of cells and tissues, provoking hyperenzymaemia. Aminotransferases are of particular clinical and diagnostically significance. Data about the blood enzyme activity of L–aspartate 2–oxoglutarate aminotransferase (ASAT), L-alanine 2–oxoglutarate aminotransferase (ALAT), gamma glutamyltransferase (gamma-GT) and alkaline phosphatase in Lacaune sheep from different parities are presented in Table 3. Regardless of lactation number, serum enzymes were within the physiological ranges. Slavov (2013) determined significant increase in ASAT, ALAT and AP as indirect parameters of normal liver functions, despite the higher tested dietary lipid levels.

Our haematological investigations in Lacaune ewes in different lactations showed statistically significant increase

Table 3
Blood serum enzymes in Lacaune ewes during the first and second lactation

Lactation	Blood serum enzynes		
	n	x	±Sx
		ASAT (U/I)	
First lactation	13	144.46	8.47
Second lactation	13	161.62	20.97
		ALAT (U/I)	
First lactation	13	16.85	1.2
Second lactation	13	18.08	1.52
		Gamma-GT (U/I)	
First lactation	13	58.15	1.66
Second lactation	13	61.38	3.18
		Alkaline phosphatase (U/I)	
First lactation	13	405.77	39.19
Second lactation	13	388.92	33.32

in leukocyte counts, haemoglobin, haematocrit, platelets, plateletcrit during the second lactation. All other studied parameters (RBC, MCV, MCH, MCHC, RDW, MPV, PDW, ASAT, ALAT, GGT, AP, blood glucose, creatinine, urea, total cholesterol, triglycerides, total protein, albumin and globulins) changed only slightly. This is in line with other studies performed on sheep (Gupta et al., 2008).

Conclusions

The analysis of results allowed concluding that:

1. As lactation number increased, along with increased milk yield, leukocyte counts ($p<0.001$) and platelets ($p<0.05$) were also increased, as well as haemoglobin content ($p<0.05$), haematocrit ($p<0.01$) and platelet mass in blood a.k.a. plateletcrit ($p<0.01$) of Lacaune ewes.
2. No changes were found out in blood total protein, albumin and globulins, which remained within the reference ranges regardless of lactation number.

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