Bulgarian Journal of Agricultural Science, 28 (No 2) 2022, 324–330

Blood biochemical profile as an objective measure of welfare in Lacaune sheep

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Abstract

Nedeva, I., Slavov, T., Radev, V., Panayotov, D. & Varlyakov, I. (2022). Blood biochemical profile as an objective measure of welfare in Lacaune sheep. *Bulg. J. Agric. Sci., 28 (2)*, 324–330

Blood serum biochemical parameters of 32 Lacaune sheep divided into two groups by productivity (high- and low-producing) were studied. The surveys were conducted in three different seasons (summer, autumn and winter). An increase ($p < 0.001$) in blood serum urea was found during the summer compared to winter in both study groups. The total cholesterol in both study groups increased ($p < 0.05$) in the autumn compared to the summer. During the autumn, triglycerides increased ($p < 0.01$-$p < 0.001$) in low- and high-producing Lacaune sheep. A decrease ($p < 0.001$) in the level of AST was found in summer and autumn compared to winter in both studied groups of sheep. GGT levels decreased ($p < 0.01$) during the summer season in low-yielding sheep compared to the winter. In high-producing Lacaune sheep, the blood serum ALP decreased ($p < 0.01$) during the summer compared to the other two seasons. The total protein in the blood serum was not affected by the season in both groups of sheep. The studied blood serum biochemical parameters in Lacaune sheep were an objective factor for their welfare.

Keywords: sheep; welfare; season; urea; total cholesterol; blood serum enzymes

Introduction

In modern livestock husbandry, the role of decision making with regard to appropriate strategy for process management with proper subsequent monitoring is essential. Thus, apart from providing the necessary conditions according to biological requirements of animals (good welfare), economic losses are also reduced as a results of elimination of the effect of stressors (Macias-Crus et al., 2016). This could be achieved through registration of physiological parameters (Maia et al., 2014) among which the most important are body temperature (Piccione et al., 2013; Martello et al., 2016), haematological profile (Silva et al., 2011) and blood hormonal profile (Koluman & Daskiran, 2011). All these physiological parameters may change either individually or in combination, particularly in animals with more primitive mechanism of adaptation to environmental changes (Beatty et al., 2006; Pantoja et al., 2017).

Sheep are small ruminants with specific characteristics compared to other farm animal species. They are better adapted to different production systems, are outlined with short generation cycles and reproductive norms leading to highly efficient production. The evaluation of physiological state is essential for development of appropriate sheep production systems. The haematological profile is part of physiological variables that could provide valuable information for health and welfare of ruminants as factors of productive performance (Bezarra et al., 2017). In ruminants, physiological parameters depend on numerous factors, such as physiological condition, age, feeding, sex, production systems

(Bruska-Jastrzębska et al., 2007; Botezatu et al., 2014), season (Farooq et al., 2017) etc. The effect of seasonal variations has been largely investigated in different animals species as dairy cattle (Abdelatif & Alameen, 2012; Casella et al., 2013; Vallejo-Timaran et al., 2020), sheep (Rathwa et al., 2017) and goats (Banerjee et al., 2015).

Biochemical parameters are responsible for the proper body function, and their deficiency leads to damage and causes structural and physiological abnormalities. The knowledge on physiological values of haematological parameters is essential for understanding systemic processes. The determination and monitoring of metabolic profile parameters may indicate whether homeostatic mechanisms are capable to maintain blood composition of animals within the physiological ranges under different conditions of rearing (Prodanovic et al., 2012). The change in blood biochemical parameters is possible indicators for prediction of sheep resistance potential to various climatic conditions and stress factors that impede their welfare.

The aim of the present study was to determine blood serum biochemical parameters as objective measure of welfare of sheep reared in intensive production systems.

Material and Methods

Study area

The study was carried out at the elite Lacaune nucleus sheep farm located in Saedinenie, district of Plovdiv. Currently, the farm raises 1800 ewes from the elite dairy breed Lacaune. Sheep are kept indoor in stalls with year-round production. The farm has 9 main facilities, 5 of which are for housing ewes. The type and architectural design of premises was similar to that of facilities for rearing Lacaune sheep in Greece. Three hundred and sixty sheep were housed in each premise. The technological solutions related to premise type at the farm were compliant with requirements of animal welfare and comfort and Ordinance 44 of April 20, 2006.

Feeding at the farm was done with total mix ration on a drive-through feeder. Feed was offered twice daily with a Feed mixer. All premises were equipped with lick blocks containing salt, micro- and macroelements.

Experimental groups of sheep

To fulfill the study goal, 32 dairy Lacaune sheep were observed during three different seasons (summer, autumn and winter). The animals were divided into 2 groups according to their productivity on the basis of data on milk yields for the preceding lactation obtained from the DeLaval DelPro management software of the farm. Ewes whose minimum milk yield was below 1.615 L were determined as low-producing whereas those with minimum milk yield over 2.143 L: as high-producing. During the experiment, all animals were fed rations offered twice daily with composition listed in Table 1.

Table 1. Composition of TMR fed to sheep during the studied seasons

Feeds	Summer, kg	Winter, kg	Autumn, kg
Alfalfa haylage	2	0.0	1
Alfalfa hay	0.8	0.6	1.2
Meadow hay	0.5	0.5	0.0
Alfalfa granules	0.6	0.0	0.0
Barley	0.4	0.5	0.0
Barley straw	0.0	0.6	0.0
Granulated sugar beet	0.225	0.0	0.0
Wheat straw	0.0	0.0	0.5
Concentrated mix	0.0	0.0	0.5
Ground corn	0.0	0.0	0.1
Total	5.44	2.2	3.3

Collection of blood samples and analysis

The analysis of blood biochemical parameters is a standard method for detection of stress, systemic metabolic changes and recently, for evaluation of sheep welfare in intensive production systems. Blood samples were collected from *v. jugularis externa* immediately after milking by a licensed veterinarian in vacutainers. Samples were assayed in a licensed laboratory to the Mobile Service Centre, University Veterinary Hospital at the Trakia University – Stara Zagora. For detection of the presence of stress and sheep welfare evaluation, the following blood biochemical parameters were assayed: glucose, creatinine, urea, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase, total protein, albumin (ALB) and globulins (GLB). Parameters were analyzed on an automated BS-120 biochemical analyzer.

Statistical analysis

Data processing and analysis was done with a standard software configures in MS Excel environment created and adapted to the study goal. All parameters are presented as mean and group standard error of means.

Results

Table 2 presents blood biochemical parameters during the studied seasons. No significant differences in blood glucose between both studied groups were found. Its concentrations demonstrated a slight increase during the winter in low-producing sheep (3.12 mmol/l), yet they were within the physiological range. Blood serum creatinine varied from 61 µmol/l to 64.24 µmol/l. There were no relevant differences both with respect to sampling seasons, nor between both groups.

Statistically significantly higher ($p < 0.001$) urea concentrations up to 11.52 mmol/l were measured in the summer in high-producing sheep compared to the winter season – 7.34 mmol/l. The same relationship was observed also in low-producing sheep in winter, when mean urea level was 6.78 mmol/l and increased considerably ($p < 0.001$) to 10.93 mmol/l during the summer. Blood urea in autumn decreased ($p < 0.01$- $p < 0.05$) in both studied groups vs summer levels. Also, in both groups, blood serum urea concentrations were lower in the winter compared to autumn ($p < 0.05$). During the autumn, blood total cholesterol of low-producing sheep increased statistically significantly ($p < 0.05$) to 2.42 mmol/l vs summer and winter. In high-producing sheep, average summer blood total cholesterol was 1.58 mmol/l and increased significantly ($p < 0.05$) to 2.5 mmol/l in winter. In high-producing sheep, blood triglycerides increased ($p < 0.001$) to attain 0.38 mmol/l in autumn compared to summer average value of 0.21 mmol/l. Blood triglycerides of low-producing sheep increased ($p < 0.01$) to 0.31 mmol/l in the autumn, whereas in the summer decreased to 0.18 mmol/l. Comparing the levels during the autumn to those in the winter, triglycerides in blood were significantly elevated in low-producing ($p < 0.05$) and in high-producing sheep ($p < 0.001$). This parameter varied also among the groups. In high-producing sheep, the average level of triglycerides was higher ($p < 0.05$) compared to low-producing sheep (0.21 mmol/l and 0.18 mmol/l, respectively).

Table 2. Blood biochemical parameters of sheep

Season	Low-producing			High-producing		
	n	**x**	**±S$_x$**	n	**x**	**±S$_x$**
GLU mmol/l						
Summer	15	2.83	0.13	17	2.86	0.07
Autumn	15	2.64	0.21	17	2.68	0.19
Winter	15	3.12	0.15	17	2.97	0.1
CREA µmol\l						
Summer	15	61	2.31	17	64	2.06
Autumn	15	61.13	4.71	17	64	4.27
Winter	15	62.13	1.88	17	64.24	1.59
UREA mmol/l						
Summer	15	10.93***	0.41	17	11.52***	0.44
Autumn	15	8.78^{a}	0.68	17	9^{aa}	0.6
Winter	15	6.78^{b}	0.29	17	7.34^{b}	0.28
CHOL mmol/l						
Summer	15	1.53	0.09	17	1.58	0.08
Autumn	15	2.42^{a}	0.27	17	2.5^{a}	0.24
Winter	15	1.66^{b}	0.1	17	1.94	0.17
TG mmol/l						
Summer	15	0.18	0.01	17	0.21c	0.01
Autumn	15	0.31^{aa}	0.04	17	0.38^{aaa}	0.04
Winter	15	0.22^{b}	0.02	17	0.17^{bbb}	0.02

* – Statistically significant differences between summer and winter seasons;
a – Statistically significant differences between summer and autumn seasons;
b – Statistically significant differences between autumn and winter seasons;
c – Statistically significant differences between low-producing and high-producing;
*, a, b, c – ($p < 0.05$); **, aa, bb, cc – ($p < 0.01$); ***, aaa, bbb, ccc – ($p < 0.001$)

Data about activity of L-aspartate:2-oxoglutarate aminotransferase (AST), L-alanine: 2-oxoglutarate aminotransferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) in Lacaune sheep during the summer, autumn and winter are presented in Table 3. During the summer, AST activity in tested low-producing sheep was 55.47 U/L on the average and increased ($p < 0.001$) to 106.07 U/L in winter. Similar results were found out during the autumn in AST activity – it increased significantly ($p < 0.001$) to 98.8 U/L vs summer levels. In high-producing sheep, the same tendency for increased

Table 3. Blood liver enzymes in sheep

Season	Low-producing			High-producing		
	n	**x**	**±S$_x$**	n	**x**	**±S$_x$**
ASAT U/L						
Summer	15	55.47^{cc}	4.17	17	39.35	3.95
Autumn	15	98.8^{aaa}	9.17	17	104.18^{aaa}	8.96
Winter	15	106.07***	12.37	17	102.65***	11.69
ALAT U/L						
Summer	15	17.67	0.86	17	17.71	0.64
Autumn	15	18.47	1.59	17	18.06	1.63
Winter	15	15.73	1.12	17	16.53	1.14
GGT U/L						
Summer	15	28.67	3.26	17	46.18^{ccc}	3.15
Autumn	15	52.67^{aaa}	4.54	17	59.77^{a}	4.84
Winter	15	57.87**	9.45	17	51.59	4.11
ALP U/L						
Summer	15	102.93	20.14	17	98.94	14.63
Autumn	15	192.07^{a}	32.95	17	206.59^{aa}	27.82
Winter	15	178.67*	31.08	17	171.53**	16.84

* – Statistically significant differences between summer and winter seasons;
a – Statistically significant differences between summer and autumn seasons;
b – Statistically significant differences between autumn and winter seasons;
c – Statistically significant differences between low-producing and high-producing;
*, a, b, c – ($p < 0.05$); **, aa, bb, cc – ($p < 0.01$); ***, aaa, bbb, ccc – ($p < 0.001$)

blood levels compared to the summer was observed both in autumn ($p < 0.001$) – up to 104.18 U/L, as well as in winter ($p < 0.001$) – up to 102.65 U/L. There were no consistent differences in blood serum ALT activity neither in relation to the season nor between the groups. Summer GGT activity in high-producing sheep was 46.18 U/L and increased substantially during the autumn ($p < 0.05$). In the summer, serum GGT activity of low-producing sheep was 28.67 U/L then increased statistically significantly to an average winter value of 57.87 U/L ($p < 0.01$). In these sheep, GGT activity in the autumn was significantly higher (52.67 U/L; $p < 0.001$) in comparison to summer values. The between-group comparison of serum GGT activities demonstrated values of 28.67 U/L in low-producing vs 46.18 U/L in high-producing sheep ($p < 0.001$). Blood ALP in low-producing sheep was 102.93 U/L and in autumn, increased significantly ($p < 0.05$) to 192.07 U/L. In winter, ALP attained 178.67 U/L – a statistically significantly higher average activity than that in the summer. In high-producing sheep, ALP activity in blood was elevated ($p < 0.01$) both in autumn and winter compared to summer.

Blood serum total protein, albumin and globulins of tested sheep are presented in Table 4. Total protein in both studied group's ranges from 70.35 g/l to 76.61, with highest concentrations during the summer. Blood serum albumin varied within 35.15-39.53 g/l in low-producing and within 34.71-38.72 g/l in high-producing sheep. Globulins of studied sheep varied from 34.94 to 37.18 g/l. Regardless of observed increases in total protein, albumin and globulins both among the seasons and between both groups, the differences were not statistically significant.

Table 4. Blood serum total protein, albumin and globulins in sheep

Season	Low-producing			High-producing		
	n	**x**	$\pm S_x$	n	**x**	$\pm S_x$
TP g\l						
Summer	15	76.61	1.53	17	74.32	0.87
Autumn	15	70.35	5.32	17	70.87	4.72
Winter	15	74.81	2.36	17	73.71	2.03
ALB g\l						
Summer	15	39.53	0.43	17	38.72	0.54
Autumn	15	35.15	2.72	17	34.71	2.39
Winter	15	39.27	0.71	17	38.7	0.93
GLB g\l						
Summer	15	37.18	1.57	17	35.82	0.6
Autumn	15	35.29	2.93	17	35.45	2.38
Winter	15	36.38	1.61	17	34.94	1.43

Discussion

Many researchers reported that blood serum glucose concentrations increased substantially in sheep under heat stress (Ellamie, 2013; Rashid et al., 2013; Sejian et al., 2013). The decreased blood glucose in sheep during the summer was probably due to reduced intake of nutrients (Nazif et al., 2003; Ramana et al., 2013; Indu et al., 2014). Karthik et al. (2021) have investigated the effect of various production systems and seasons on sheep blood parameters. They found out that in extensively reared sheep, serum glucose was lower when they grazed on pasture for 8 hours per day (Kulkarni et al., 2010; Kochewad et al., 2018). Heat stress increases insulin concentrations in the circulation, which is of primary significance for glucose uptake in peripheral tissues (Min et al., 2015). The lower availability of substrate for synthesis of glucose in a large system is another cause for lower serum glucose concentrations. When an animal is exposed to various stress factors, blood cortisol level increases consequently to the stimulation of hypothalamic-pituitary-adrenal axis. Increased cortisol levels may results in higher blood sugar concentrations. What is more, the enhanced sympathoadrenal activity, stimulated by physiological and psychological stress may also result in hypoglycaemia through liver glycogen degradation (Adenkola & Ayo, 2010; Ali et al., 2006). Other research studies on blood parameters of Lacaune sheep (Slavov et al., 2018) reported increased blood glucose during the first lactation vs the second one due to increased milk production and lactose release in milk. Antunovic´ (2002) observed a seasonal effect on reduced blood glucose levels. The addition of Sargassum latifolium to the diet of lambs subjected to heat stress reduced considerably blood serum glucose and lipid profile parameters (Ibrahim et al., 2020). Despite the presented increase in glucose concentrations, statistically significant seasonal and between-group variations were not proved.

Blood urea and creatinine concentration in animals are important tools in the diagnosis of diseases, some metabolic disturbances and evaluation of renal glomerular filtration (Kour et al., 2014). Normal creatinine levels in the blood of sheep vary between 44-150 µmol/l and increased when glomerular filtration is compromised. Varlyakov et al. (2015) found slightly increased blood creatinine in yearling sheep whose ration was supplemented with Optigen, both before and after feeding, yet differences were insignificant. Despite the recorded increase in this parameter, it remained within the reference range and did not pose any threat to animal health and welfare. Ibrahim et al. (2020) found considerable changes in the activity of kidneys, whereas blood plasma urea levels were significantly elevated ($p < 0.05$) in lambs subjected to heat stress compared to control lambs. Several research teams (Srikandakumar et al., 2003; Kour et al., 2014; Rathwa et al., 2017) reported that the observed increase in blood urea and creatinine concentrations during heat stress was due to enhanced blood flow in peripheral capillary network, reduced kidney blood flow and reduced glomerular filtration rate during heat stress. A lot of reports affirmed that serum cholesterol levels increased when sheep were continuously exposed to high ambient temperatures (Ellamie, 2013; Rashid et al., 2013; Sejian et al., 2013). Other authors attributed the lower blood cholesterol in sheep (Nazif et al., 2003; Sejian et al., 2010; Ramana et al., 2013; Indu et al., 2014) during the summer season to the reduced intake of nutrients. Having tested rations supplemented with 4% *Sargassum latifolium,* Ibrahim et al. (2020) found out that total blood cholesterol in lambs under heat stress was reduced. Kannan et al. (2007a, b) and Sucu et al. (2017) demonstrated that blood cholesterol tended to decrease in goats, dairy cows and lambs under heat stress fed diets containing algae.

The established considerably elevated activities of AST, ALT, GGT and ALP in the blood as indirect indices for the normal liver function, are probably due to the different composition of fed rations, the higher dietary protein level and the productive performance of Lacaune sheep. In the liver, L-aspartate:2-oxoglutarate aminotransferase (AST), L-alanine: 2-oxoglutarate aminotransferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) have high activity and are most commonly assayed when acute and chronic liver diseases are suspected (Steen, 2001). The results reported by Karthik et al. (2021) confirmed that the production system and the season influenced blood AST and ALT. The reported results were contrary to ours. They found out that AST and ALT were increased in sheep under heat stress. In general, increased AST concentrations are associated with liver and muscle disorders, whereas higher ALT blood activity – only to liver disturbances. Nevertheless, in the present study, the obtained results may result from enhanced gluconeogenesis from dietary protein sources, thus posing a load on the liver (Kochewad et al., 2018b). According to Weigand et al. (2007), higher serum AST or ALT activities in every species are associated with decreased tolerance to heat. It was demonstrated that serum aminotransferases are sensitive markers of tissue damage and that increased serum levels are indicative for cell leakage and loss of the functional cell membrane integrity (Brancaccio et al., 2010; Khan et al., 2013). Contrary to our results, a lot of other studies indicate that the serum activity of AST, ALT and ALP increased in sheep during the summer season, when they suffer from heat stress (Cwynar et al., 2014; Aleena et al., 2016).

Total protein and albumin concentrations in blood of sheep reared in intensive production systems was higher ($p < 0.001$) in the winter, whereas blood globulins showed the opposite tendency (Karthik et al., 2021). Higher serum total protein in intensively reared sheep may result from increased efficiency of conversion of non-protein nitrogen compounds into amino acids and proteins. Intensively reared sheep may also have higher rumen microbial and infusorial concentrations, which synthesize protein from available non-protein nitrogen and urea-containing rations (Reddy et al., 2019 a, b).

Conclusions

The analysis of blood biochemical parameters as objective measure of sheep welfare in intensive production systems allowed concluding that: although data for blood biochemical parameters were comparable to respective reference values for sheep, the season and the production system influenced their blood biochemical profile. Blood biochemical parameters that were statistically significantly influenced by the season were: urea, total cholesterol, triglycerides, AST, GGT and alkaline phosphatase. The present study may be useful as a source of information about blood biochemical parameters in dairy sheep with regard to evaluation of their welfare and physiological condition.

Acknowledgments

This work was supported by the project „Strengthen Research and Innovation Capacity for Grazing Livestock Meat Production in Bulgaria through Advanced Knowledge Transfer“, „Greenanimo“ (contract No Д01-287/07.10.2020 and Д 01-85/01.04.2021) part of the National Programme “European Scientific Networks” funded by the Ministry of Education and Science, Republic of Bulgaria.

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Received: January 4, 2022; *Accepted:* February 11, 2022; *Published:* April, 2022