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STUDY REGARDING THE VARIATIONS OF SOME HAEMATOLOGIC, ELECTROLYTIC, ENZYMATIC AND BIOCHEMICAL PARAMETERS IN EARLY LACTATION IN EWES

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Abstract

The aim of this study is to highlight the influence of early lactation physiological stage on the haematological, electrolytic, enzymatic and biochemical profiles in ewes. The studied parameters (WBC, RBC, Hb, Ht, MCV, MCH, MCHC, Ca, P-inorganic, K, Na, Cl, ALT, AST, CK, GGT, LDH, glucose, urea, cholesterol, triglycerides, creatinine, total proteins, albumin, total bilirubin) were determined in the 20th day of the lactation period, the results obtained being compared to those of the witness group of non-pregnant nor lactating ewes. Important variations of the studied parameters were observed. The WBC value (7.21 ± 0.29 m/mm^3) decreased significantly ($p<0.05$); the calcium and phosphorus values (9.20 ±2.56 ;4.25± 0.60 mg/dL) decreased non-significantly ($p>0.05$); the AST (21.60 ± 2.41 U/L) and GGT (12.10 ± 1.45 U/L) activity increased significantly ($p<0.05$); the glucose (66.1 ± 8.43 mg/dL) and cholesterol (47.90 ± 7.01 mg/dL) values decreased significantly ($p<0.05$). The results are correlated with the high energy demand in order to support the mammary gland intense activity of milk synthesis in the first third of the lactation period.

Key words*: biochemical parameters, ewes, haemoleucogram, lactation.*

INTRODUCTION

Ewes' breeding is closely correlated with the productive and reproductive potential, the outstanding adaptability and resistance to the various environmental conditions and the superior bio economical characteristics of this species. In order to increase the profitability and efficiency of the ewes' exploitation, it is necessary to know each individual's necessities imposed by the physiological stage in which it is found.

In this context, the haematology and blood biochemistry analyses allow monitoring the animal health status in various physiological stages. The importance of the main blood parameters determination is highlighted by the fact that the wide variations of their mean values may cause disturbances of the homeostasis and, implicitly, systemic disorders, even if the animal's general condition remains unchanged (Crivineanu et al., 2010; Turcitu et al., 2012).

The first third of lactation period, considered by most authors the interval between the first and the 60th day of lactation, as the average lactation period in ewes is about 180 days long, represents a metabolically demanding physiological stage (Anwar et al., 2012). Thus, the main objective of this study was monitoring a series of blood parameters (haematological, electrolytic, enzymatic and biochemical) in the 20th day of lactation in order to reveal their fluctuations, therefore the influence of this physiological stage, the research being also focused on two main aspects: maintaining the ewes' health and maintaining a high level of productivity.

MATERIALS AND METHODS

The study was carried out between August 2017 and April 2018 in a livestock of ewes belonging to a household in the village of Comosteni, Dolj County. The experimental group consisted of 10 healthy individuals, with no history of pathologies, approximately equal body mass and multiparous.

The blood sampling was performed on: August 15, 2017 for non-pregnant ewes and February 25, 2018 for lactating ewes, on the 20th day of lactation. The haematological parameters were determined by using whole blood and the MS-45™ haematology analyser (Melet Schloesing

Laboratoires, France). The biochemical and enzymatic parameters were determined by using plasma and the Spotchem EZ 4430 ARK RAY analyser. For the electrolytic parameters, plasma was collected in sterile syringes, which were then labelled, refrigerated and dispatched to a commercial laboratory according to the standard laboratory procedures, the samples being processed by automatic analysers using the spectrophotometric (colorimetric) method.

RESULTS AND DISCUSSIONS

Haematological profile

The haematological parameters variations in the first third of the lactation period were predominantly not significant from a statistic point of view ($p>0.05$) and the mean values shown in Table 1 and Figure 1 were found within the limits of the reference ranges of this species for all studied parameters.

Table 1. The haematological profile' mean values in non-pregnant ewes and in the 20th day of the lactation period

PARAMETER	PHYSIOLOGICAL STATUS	
	Non-pregnant	20th day of lactation
WBC (m/mm^3)	8.32 ± 0.44	7.21 ± 0.29**
RBC (M/mm^3)	11.04 ± 0.77	10.17 ± 1.38*
Hb (g/dL)	12.07 ± 1.11	11.20 ± 0.94*
Ht (%)	36.1 ± 3.32	34.3 ± 2.95*
MCV(fl)	32.7 ± 2.11	33.7 ± 2.01*
MCH (pg)	10.9 ± 0.78	11.0 ± 0.57*
MCHC(g/dL)	33.4 ± 2.01	32.6 ± 1.97*

*p>0.05 - statistically non-significant differences
**p<0.05 - statistically significant differences

The analysis of the main haematological parameters (WBC, RBC, haemoglobin, haematocrit, MCV, MCH, MCHC) in correlation with the physiological status in ewes revealed that in the 20th day of the lactation period can be observed a statistically significant decrease ($p<0.05$) of the WBC mean value and a non-significant decrease ($p>0.05$) of the RBC and haemoglobin compared to the mean values obtained in non-pregnant ewes, that also represented the control group. Other studies regarding the haematological parameters in sheep or cattle reported similar results, the main cause incriminated by most authors being the migration of the leukocytes from blood to milk in order to protect the mammary gland from the pathogens' action by phagocytosis, but also in order to provide the necessary load of antibodies for the new-born lamb (Antunovic et al., 2011). The high concentration of white blood cells in ewes' milk was demonstrated and highlighted using milk cytological investigations in other research studies (Ognean et al., 2016). Also, a RBC mean value ($p>0.05$) decrease was observed, this results being consistent with those recorded by other authors and mentioned in the specialized literature. MCV, MCH and MCHC remained unchanged throughout the study not showing any statistically significant variations ($p>0.05$).

The overall results regarding the haematological profile are in accordance with various studies on this topic, present in the speciality literature (Anwar et al., 2012; Khaled. & Illek, 2012; Bamerni, 2013).

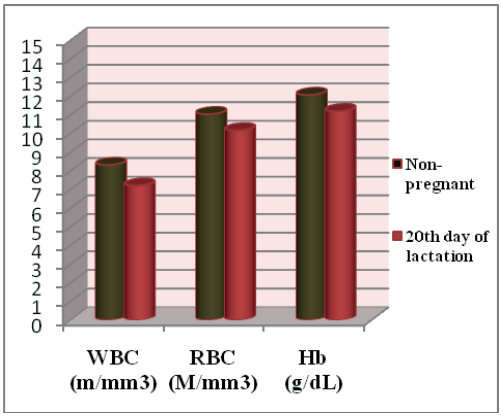

Figure 1. The dynamics of the WBC, RBC and Hb mean values in non-pregnant ewes and in the 20th day of the lactation period

The variations of the haematological parameters observed in the first third of the lactation period, respectively on the 20th day of lactation in ewes, were predominantly not significant from a statistical point of view ($p>0.05$). The analysis of the haematological parameters in correlation with the physiological status revealed that at the beginning of the lactation period, there can be observed a significant decrease of the mean values of the white blood cells count ($p<0.05$) and a non-significant decrease ($p>0.05$) of the mean

values of erythrocytes count and haemoglobin concentration compared to the mean values obtained in non-pregnant ewes. The main cause of the white blood cells count mean values significant decrease in the 20th day of lactation (*p>0.05*) was the massive migration of the leukocytes from the mother's blood into milk in order to provide the necessary antibodies for the new-born lamb and also to protect the mammary gland by phagocytosis.

Electrolytic profile

There have been observed important variations of the electrolytic parameters mean values, this findings being most likely associated with the increased milk synthesis in the early stages of lactation. The evolution of the mean values of the electrolytic profile parameters is presented in Table 2. The variations of the electrolytic parameters are considered by the speciality literature to be expected in this metabolically demanding period, in this species, not being associated, normally, with pathological stages (Codreanu, 2016).

Table 2. The electrolytic profile' mean values in non-pregnant ewes and in the 20th day of the lactation period

PARAMETER	PHYSIOLOGICAL STATUS	
	Non-pregnant	20th day of lactation
Ca (mg/dL)	9.45 ± 2.72	9.20 ± 2.56*
P-inorganic (mg/dL)	4.31 ± 0.64	4.25 ± 0.60*
K (mmol/L)	5.98 ± 0.49	6.28 ± 0.69*
Na (mmol/L)	153.97 ± 2.09	159.70 ± 2.15*
Cl (mmol/L)	102.55 ± 3.41	117.30 ± 0.95*

*p>0.05 – statistically non-significant differences

The decrease of the calcium blood concentration in ewes after parturition, at the beginning of the lactation period can be associated with the increased calcium secretion through milk and, implicitly, its rearrangement in bones (Liesegang et al., 2007). Calcium and phosphorus are mobilized in similar ways, but larger quantities of calcium are excreted through milk. Phosphorus isn't secreted in milk in the same amount as calcium, therefore its blood concentration being higher.

The potassium mean values increased non-significant (*p>0.05*) in the 20th day of the lactation period compared with the results obtained in non-pregnant ewes. The fluctuations of the main electrolytes concentrations, especially potassium, may lead to structural and functional imbalances and also to tissues and organs dysfunctions. For example, skeletal and cardiac muscle dysfunctions may occur when the potassium level fluctuates, therefore maintaining a constant potassium level is necessary in order to maintain the homeostasis.

The electrolytic parameters variations in the first third of the lactation period, respectively on the 20th day of lactation, are associated with the increased milk synthesis and their excretion through milk.

Enzymatic profile

The blood enzymes activity of the studied group of ewes is presented in Table 3 and Figure 2. All the studied enzymatic parameters fell between the reference range values, except for the GGT activity that slightly exceeded the upper limit of the reference interval in the 20th day of lactation. Significantly elevated concentrations of AST and GGT were detected in lactating ewes compared to not pregnant ewes ($p<0.05$). An opposite trend - decreasing - was observed in the ALT concentration but with no statistically significant differences ($p>0.05$).

The significant increase of the AST and GGT activity in lactating ewes indicates, most likely, an increase of the hepatic metabolism (Codreanu & Călin, 2018). The results are consistent with those recorded in various other studies regarding the influence of the physiological status on the enzymatic activity in ewes and cattle (Antunovic et al., 2011; Anwar et al., 2012; Caldeira et al., 2007). The high AST values observed on the 20th day of lactation when the highest milk production is expected to occur indicate a stimulation of the liver function, phenomenon associated most of the time with high productivity periods.

CK mean activity showed a moderate increase but with no statistically significant variations ($p>0.05$).

LDH activity was also visibly increased at the beginning of the lactation period, but with no statistically significant differences (*p>0.05*).

All the enzymatic variations are due to an intense liver activity in lactating ewes, in order

to sustain an increased energy and protein demand imposed by the milk production.

Table 3. The enzymatic profile' mean values in non-pregnant ewes and in the 20th day of the lactation period

PARAMETER	PHYSIOLOGICAL STATUS	
	Non-pregnant	20th day of lactation
ALT (U/L)	19.90 ± 2.58	16.44 ± 3.73*
AST (U/L)	15.30 ± 3.74	21.60 ± 2.41**
CK (U/L)	87.50 ± 7.19	84.20 ± 6.28*
GGT (U/L)	8.88 ± 2.31	12.10 ± 1.45**
LDH (U/L)	412.50 ± 44.75	486.60 ± 69.19*

*p>0.05 – statistically non-significant differences
**p<0.05 – statistically significant differences

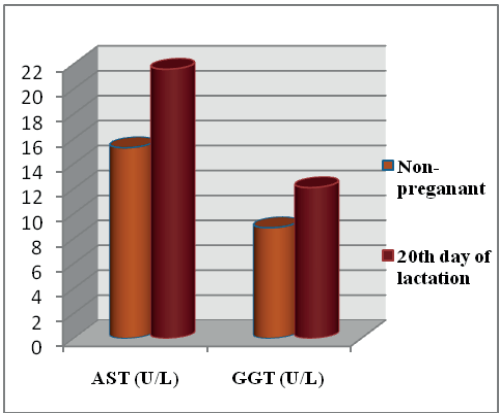

Figure 2. The dynamics of the AST and GGT activity in non-pregnant ewes and in the 20th day of the lactation period

The mean blood enzymatic activity of the studied group of ewes intensified in the first third of the lactation period. The mean activity of AST showed a significant increase ($p<0.05$) at the start of the lactation period, not exceeding the upper limit of the reference range. GGT activity also increased significantly ($p<0.05$) in the 20th day of the lactation period, even exceeding the upper limit of the reference range for this species. The enhanced enzymatic activity indicates an intensification of the hepatic functions in lactating ewes, in order to support the milk production and, implicitly, the increased energy and protein demand.
The overall results obtained in the enzymatic profile review indicate that no pathological entity was involved in this case, according to the speciality literature, the variations of the enzymatic profile being associated with the physiological stage, not with a pathological event (Codreanu, 2016).

Biochemical profile

The mean values of the studied biochemical parameters are shown in Table 4 and Figure 3.
There have been observed important variations of the studied parameters, their mean values generally fell within the range of the reference values for this species. Variations of the blood levels of some biochemical indicators such as glucose, cholesterol, triglyceride and urea - indicate an energy shortage in ewes at the beginning of the lactation period.

Table 4. The biochemical profile' mean values in non-pregnant ewes and in the 20th day of the lactation period

PARAMETER	PHYSIOLOGICAL STATUS	
	Non-pregnant	20th day of lactation
Glucose (mg/dL)	90.3 ± 13.03	66.1 ± 8.43**
Urea (mg/dL)	21.00 ± 2.80	23.77 ± 3.11*
Cholesterol (mg/dL)	61.90 ± 8.96	47.90 ± 7.01**
Triglycerides (mg/dL)	10 ± 1.34	10.5 ± 1.51*
Creatinine (mg/dL)	0.88 ± 0.09	0.78 ± 0.05*
Total proteins (g/dL)	6.73 ± 0.46	5.90 ± 0.31*
Albumin (g/dL)	3.41 ± 0.38	2.80 ± 0.31*
Total bilirubin (mg/dL)	0.20 ± 0.02	0.41 ± 0.09**

*p>0.05 – statistically non-significant differences
**p<0.05 – statistically significant differences

Glucose mean values decrease statistically significant ($p<0.05$) on 20th day of lactation as compared to the values obtained in not pregnant ewes. The decrease of this parameter's mean values at the onset of the lactation period can be the result of the constant loss of energy due to the increased milk production. These changes suggest that the intensive use of glucose for the lactose synthesis in the early lactation period may be incriminated for the body's inability to maintain the glucose homeostasis. The statistically significant ($p<0.05$) decrease of the glucose concentration may also be associated with the increased milk production during the

first part of the lactation period and with the intense activity of the mammary gland.

The slightly elevated urea levels ($p>0.05$) in lactating ewes may be caused by the mobilization of large amounts of body reserves, translated into an intense muscle protein catabolism.

Decreased total protein and albumin concentrations ($p>0.05$) can be a consequence of the intense mobilization of plasma immunoglobulin in order to form the colostrum in the mammary gland, this process taking place during the last months of gestation.

The mean values of cholesterol decreased significantly ($p<0.05$), being consistent with the increased energy demand in the first third of the lactation period. The slightly increase of the triglycerides mean value ($p>0.05$) in early lactation can be associated with an increased mobilization of fat from the adipose tissue due to the negative energy balance in this period.

There was also observed a significant increase ($p<0.05$) of the total bilirubin mean value, indicating an intensification of the hepatic metabolism, along with the results obtained in the enzymatic profile parameters.

The mean value of the blood creatinine showed little variations that were not statistically significant ($p>0.05$).

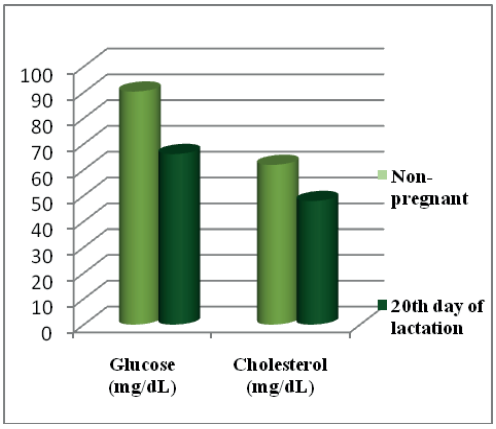

Figure 3. The dynamics of the glucose and cholesterol mean values in non-pregnant ewes and in the 20th day of the lactation period

The results obtained in the study of the biochemical parameters are correlated with the high energy demand in this physiological stage. Therefore, the glucose mean values decreased significantly ($p<0.05$) on the 20th day of lactation as a result of the increased energy loss due to milk synthesis and intense activity of the mammary gland. These changes suggest that the increased utilization of the glucose for the synthesis of lactose during the first third of the lactation period can lead to the body's inability to maintain the glucose homeostasis. Slightly elevated levels of urea in lactating ewes may be a consequence of the muscle protein catabolism intensification when large amounts of body reserves are mobilized. The decreased albumin and total protein mean values on the 20th day of lactation are due to the intense mobilization of plasma immunoglobulin during the last months of gestation when the colostrum is formed in the mammary gland.

CONCLUSIONS

The variations of the studied parameters in the first third of the lactation period in ewes indicate an energy deficiency and an important intensification of the metabolism overall. The analysis of the haematological parameters revealed a significant decrease of the white blood cells count mean values due to the migration of the leukocytes from the mother's blood into milk in order to provide antibodies for the new-born and for the protection of the mammary gland, results that are also correlated with the decreased albumin and total protein mean values due to the immunoglobulin's mobilization in the last months of gestation when the colostrum is formed. The variations observed in the biochemical profile were the result of the increased energy loss due to milk synthesis and mammary gland intense activity and also a consequence of the muscle protein catabolism intensification due to the increased mobilization of body reserves.

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