

INVESTIGATIONS REGARDING THE INFLUENCE OF THE PHYSIOLOGICAL STATUS ON SOME ELECTROLYTIC, ENZYMATIC AND BIOCHEMICAL BLOOD PARAMETERS IN EWES

INVESTIGAȚII PRIVIND INFLUENȚA STATUSULUI FIZIOLOGIC ASUPRA UNOR PARAMETRI ELECTROLITICI, ENZIMATICI ȘI BIOCHIMICI SANGVINI LA OAIE

Iuliana CODREANU^{1),*},
Simona CĂLIN²⁾

ABSTRACT | REZUMAT

Ewes' breeding in Romania has been influenced by the great organic resistance and the adaptive capacity of this species to the environmental and microclimate conditions, but also to the special nutritional and economical values of the products obtained. The aim of this research paper is to reveal the influence of the physiological status on a series of electrolytic, enzymatic and biochemical parameters (calcium, phosphorus, potassium, sodium, ALT, AST, CK, GGT, LDH, total proteins, urea, creatinine, albumin, glucose, cholesterol, triglycerides, total bilirubin), and invariably the importance of the correction and correlation of these parameters in order to maintain the health and well-being of the animals and at the same time to reach the maximum economic potential. For the purpose of this study, a group of 10 clinically healthy ewes, raised in an extensive system, were marked and followed during three stages of the physiological status: non-gestational, gestation and lactation on day 20, day 40 and day 60. Significant variations in electrolytes values have been detected during the study, which may be associated with high fetal demands due to an intensive growth rate in advanced gestation and also due to increased milk synthesis in the early stages of lactation. The blood enzyme activity of the studied ewes is generally situated between the reference limit values for all determined parameters, except for GGT that slightly exceeded the upper limit of the reference range during the lactation period. This may be due to more intense liver activity in lactating ewes, which is likely to sustain an increased energy and protein demand in order to support the milk production. Within the blood biochemical profile, important variations of the mean values for the studied parameters were found, all parameters presenting mean values that, generally, were between the limits of the reference interval for this species. Changes of the plasma concentrations of some biochemical parameters (glucose, cholesterol, triglycerides, urea) - indicate an energy deficiency in early lactating ewes. In conclusion, the studied parameters values

Creșterea ovinelor pe teritoriul României a fost încă din cele mai vechi timpuri influențată de rezistența organică deosebită și capacitatea adaptativă ridicată a acestei specii la condițiile de mediu și microclimat, dar totodată și de diversitatea, valoarea nutritivă deosebită și valoarea economică a produselor obținute.

Scopul prezentei lucrări este acela de a consemna influența statusului fiziologic asupra unei serii de parametri electrolitici, enzimatici și biochimici (calciu, fosfor, potasiu, sodiu, clor, ALT, AST, CK, GGT, LDH, proteine totale, uree, creatinină, albumine, glucoză, colesterol, trigliceride, bilibrubină totală) la oaie, și, invariabil, importanța corectării și corelării acestor parametri în vederea menținerii stării de sănătate și bunăstare a animalelor și, totodată, atingerii potențialului productiv maxim din punct de vedere economic.

Pentru realizarea acestui studiu a fost alcătuit un lot de 10 ovine clinic sănătoase, crescute în sistem extensiv, care au fost marcate și urmărite pe parcursul a 3 etape ale statusului fiziologic: negestante, gestante și în ziua 20, ziua 40, respectiv, ziua 60 de lactație. Pe durata studiului au fost observate variații marcante ale valorilor electroliților, acestea putând fi asociate cu cerințele ridicate ale fătului ce prezintă un ritm intensiv de creștere în gestația avansată și totodată datorate sintezei crescute de lapte în stadiul incipient al lactației. Activitatea enzimatică sangvină a lotului de oi luat în studiu se înscrie în intervalul de referință pentru toți parametrii enzimatici determinați, cu excepția valorii GGT care a depășit ușor limita superioară a intervalului de referință pe parcursul perioadei de lactație. Acest fapt se poate datora unei activități hepatice mai intense la oile lactante, aspect ce vine în preîntâmpinarea unui necesar energetic și proteic crescut în vederea susținerii producției de lapte. În cadrul profilului biochimic sangvin s-au constatat variații marcante ale valorilor medii pentru parametrii studiați, aceștia încadrându-se, în general, în limitele intervalelor de referință pentru această specie. Variațiile concentrațiilor plasmatiche ale unor indicatori biochimici (glucoză, colesterol, trigliceride, uree) – indică un deficit energetic la oile aflate în lactație incipientă.

În concluzie, parametrii studiați s-au încadrat, în general, în limitele intervalului de referință pentru această specie, înregistrându-se totodată fluctuații mar-

1) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania

2) The General Association of Romanian Veterinarians, Bucharest, Romania

*) Corresponding author: iulianacod@yahoo.com

were generally found within the limits of the reference range for this species, while considerable fluctuations were discovered in the parameters that indicate the energy deficit during metabolically demanding physiological periods.

Key words: physiological status, ewes, gestation, lactation, biochemical parameters

In the context of increasing the efficiency and developing the profitability of ewes' exploiting, it is necessary to know in detail the data on the metabolic profile of this species in direct correlation with the necessities imposed by the physiological status in which they are found.

From an economic point of view, the breeding of ewes is effectively correlated with the productive potential, the morpho-physiological peculiarities, the adaptability and the resistance to the various environmental conditions and the superior bioeconomical characteristics of this species.

In the last few years, most research regarding the small ruminants has mainly focused on studying the pathology and pathogens of these animals (3, 6, 10, 19) and, to a lesser extent, on the analysis of the physiological phenomena that, in some particular situations, might play an important role in maintaining the health and wellbeing of the small ruminants.

Since the last part of gestation and the first part of lactation are metabolically demanding physiological periods, by monitoring the electrolytic (calcium, phosphorus, potassium, sodium), enzymatic (ALT, AST, CK, GGT, LDH) and biochemical (total proteins, urea, creatinine, albumin, glucose, cholesterol, triglycerides, total bilirubin) parameters, the research is focused on three main aspects: maintaining the mother's health, ensuring the harmonious growth and health of the product / products of conception and keeping the productivity at the desired level.

The aim of study is to reveal the influence of the physiological status on a series of electrolytic, enzymatic and biochemical parameters (calcium, phosphorus, potassium, sodium, ALT, AST, CK, GGT, LDH, total proteins, urea, creatinine, albumin, glucose, cholesterol, triglycerides, total bilirubin) and their importance in the health and wellbeing of animals.

METHODS AND MATERIALS

Animals

The experiment has been conducted in a flock of 62 sheep and goats belonging to a household, from

cante ale parametrilor care indică deficitul energetic pe parcursul perioadelor fiziologice solicitante din punct de vedere metabolic.

Cuvinte cheie: status fiziologic, ovine, gestație, lactație, parametri biochimici

Comoșteni village, Gângiova commune, Dolj County (Romania).

Blood samples

In order to perform the biochemical, enzymatic and electrolytic analyzes, blood samples were collected in the morning, between 9 and 10 a.m., after feeding, through the holder-vacutainer system into Lithium Heparin tubes. Each tube was numbered specifically in order to correspond to each of the 10 subjects. The tubes were centrifuged for 4 minutes at 3000 rpm in order to obtain plasma which was subsequently harvested using 1 ml sterile syringes.

Design of experimental study

In the present experiment, we studied a group of 10 healthy ewes for a period of time of 9 months – from August 2017 to April 2018. The selection criteria were: the state of health, the age and the number of previous lambs/kids. Thus, the group consisted of ewes with a proper maintenance status, approximately equal body mass, clinically healthy and with no medical history of pathologies of any nature, approximate age of 3 years, multiparous, without twin gestations, or previous fetal-maternal dystocia. The 10 ewes were marked and observed during 3 stages of their physiological status, i.e. non-gestational, gestation and lactation on day 20, day 40 and day 60, respectively. Sampling at the non-gestational stage was performed on 15th August 2017. The duration of gestation was about 149 days (September 10, 2017 - February 6, 2018), and the samples from the pregnant ewes were taken on 11th December, 2017, about the 90th day of gestation. For the lactation period the samples were collected on the 20th, 40th and 60th days of the lactation, respectively on the dates: February 25, 2018, March 17, 2018 and April 6, 2018. Over the course of the study - about 9 months, the animal health status did not change, the 10 ewes did not show clinical signs of disease, there were no signs of dystocia, the fetal mortality rate was 0 % and no post-partum behavioral disorders were recorded.

Biochemical and enzymatic exams

In the interest of measuring the biochemical and enzymatic parameters, plasma was introduced into

Table 1

The mean values of the electrolytic blood profile in correlation with the physiological status of the studied group of ewes (X ± sx)

Physiological status Parameter	Non-pregnant	Pregnant	Lactating			Reference range
			20 th day	40 th day	60 th day	
Ca (mg/dL)	9.45 ± 2.72	9.41 ± 2.68*	9.20 ± 2.56*	8.75 ± 2.11**	10.02 ± 1.34*	9.3 - 11.7
P-inorganic (mg/dL)	4.31 ± 0.64	4.28 ± 0.61*	4.25 ± 0.60*	5.78 ± 0.44**	5.09 ± 0.72*	4 - 7.3
K (mmol/L)	5.98 ± 0.49	5.83 ± 0.40*	6.28 ± 0.69*	5.97 ± 0.48*	6.12 ± 0.58*	4.3 - 6.3
Na (mmol/L)	153.97 ± 2.09	144.01 ± 1.88*	159.70 ± 2.15*	145.82 ± 2.04*	159.88 ± 2.19*	142 - 160
Cl (mmol/L)	102.55 ± 3.41	112.05 ± 1.27*	117.30 ± 0.95*	112.70 ± 1.93*	112.90 ± 2.03*	101 - 113

*p > 0.05 - statistically significant difference; **p < 0.05 - statistically non-significant difference; sx - standard deviation

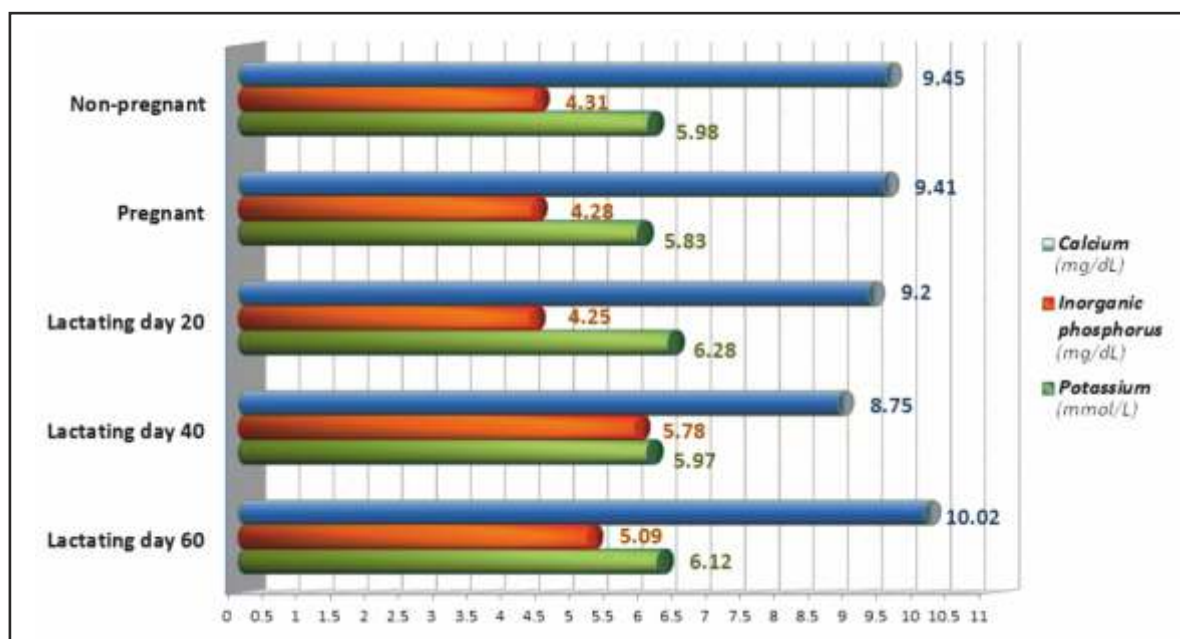


Fig. 1. Dynamics of the mean concentration values of Ca, P and K in correlation with the physiological status of the studied group of ewes

the special test tube of the Spotchem EZ 4430 ARK RAY Analyzer.

For the values measurement of the electrolyte parameters, the plasma was collected in sterile syringes, which were then labeled, refrigerated and dispatched to a commercial laboratory according to the standard laboratory procedures. The samples were processed by automatic analyzers using the spectrophotometric (colorimetric) method.

RESULTS AND DISCUSSIONS

Electrolytic profiles

There have been observed considerable variations of the electrolytic values, which may be associated with high fetal demands which has an intensive growth rate in advanced gestation and also due to increased milk synthesis in the early stages of lactation. The evolution of the mean values of the electrolytic profile pa-

rameters in correlation with the physiological status of the studied group of ewes is presented in Table 1 and in the graph of Fig. 1.

A statistically significant ($p < 0.05$) decrease in calcium concentration and a statistically non-significant decrease ($p > 0.05$) of the sodium concentration compared to the mean values obtained in non-pregnant ewes were recorded in ewes in the 40th day of lactation. In the 60th day of lactation, the mean values of the calcium and sodium presented, in both parameters, a non-significant increasing comparing to the reference values of this species. An opposite trend was observed in the inorganic phosphorus concentration, which on the 40th day of lactation showed a statistically significant increase ($p < 0.05$) compared to the mean values obtained in the case of non-pregnant ewes and then a non-significant decrease from the statistical point of view ($p > 0.05$) on the 60th day of lactation.

The decrease of calcium concentration in the blood of the ewes after parturition, but also on the lactation period can be associated with the increased calcium secretion through milk and its rearrangement in bones. Decreased calcium levels in the blood may be also caused by feeding animals with wheat bran in this period - when the animals have a restricted diet in order to prevent the excessive gas accumulation in the rumen (17).

Calcium and phosphorus are mobilized from bones in similar ways, but more calcium from the body and blood is excreted through milk. Phosphorus is also secreted in milk, but not in the same amount as calcium and therefore its blood concentration is higher.

The lowest concentrations of sodium and potassium were observed in ewes with advanced gestation, the decrease not being statistically significant ($p > 0.05$) compared to the values obtained in non-pregnant ewes. These results were in accord with Antunović et al. (2004), studies (1).

In lactating ewes, higher potassium concentrations were determined compared to pregnant ewes, with a statistically non-significant ($p > 0.05$) increasing of the mean values. Maintaining a constant potassium level is necessary in order to maintain homeostasis, since substantial fluctuations in electrolytes concentrations, especially potassium, may lead to structural and functional imbalances, such as cardiac, smooth and skeletal muscles dysfunction (29).

On the 40th day of lactation, mean values of sodium and chlorine were lower compared to the 20th day of lactation, with non-significant differences ($p > 0.05$). The reason could be the increased excretion of electro-

lytes through milk, due to the increased milk production during this specific lactation period (20).

Enzymatic profiles

The blood enzyme activity of the studied group of ewes is presented in Table 2 and Fig. 2 and was found between the reference range values for all determined parameters, except for the GGT activity that slightly exceeded the upper limit of the reference interval during the lactation period. This may be due to a more intense liver activity in lactating ewes, which is likely to sustain an increased energy and protein demand in order to support the milk production (23).

Significantly elevated concentrations of AST and GGT were detected in lactating ewes compared to pregnant and not pregnant ewes ($p < 0.05$).

An opposite trend - decreasing - was observed in the ALT concentration in lactating compared to pregnant and not pregnant ewes but without statistically significant differences ($p > 0.05$). The increase in AST and GGT activity in lactating ewes indicates an increase of the hepatic metabolism. The results obtained in this study are consistent with those previously reported by Antunović et al (2004) (1).

The highest AST values were observed in ewes on the 20th day of lactation when the highest milk production is expected to occur. Increased AST activity indicates a stimulation of the liver function - a fact associated with high productivity.

CK activity did not show statistically significant variations ($p > 0.05$) in correlation with the physiological status, although a moderate increase of its values was observed during lactation.

Related to the values obtained in non-pregnant ewes, LDH activity is visibly increased during lactation but with no statistically significant differences ($p > 0.05$). On the 40th day of lactation, there was recorded a decrease of the LDH activity compared to the 20th day of lactation but without statistically significant variations compared to the results obtained in not pregnant ewes ($p > 0.05$).

Changes in the activity of these enzymes may also be associated with reduced intake of dry fodder near parturition, which can lead to hepatic lipidosis and therefore to an altered liver function (14).

Biochemical profiles

The results of the blood biochemical profile of the non-pregnant, pregnant, lactating on day 20, 40 and 60 ewes are shown in the synthetic Table 3 and the graphs in Fig. 3 and Fig. 4. There have been noticed significant variations, all parameters presenting mean values that generally fall within the range of the re-

Table 2

The mean values of the enzymatic blood profile in correlation with the physiological status of the studied group of ewes (X ± sx)

Physiological status Parameter	Non-pregnant	Pregnant	Lactating			Reference range
			20 th day	40 th day	60 th day	
ALT (U/L)	19.90 ± 2.58	19.70 ± 3.84*	16.44 ± 3.73*	16.80 ± 3.44*	17.50 ± 2.37*	8 - 57
AST (U/L)	15.30 ± 3.74	14.66 ± 3.32*	21.60 ± 2.41**	18.20 ± 3.62*	17.55 ± 3.42*	9 - 49
CK (U/L)	87.50 ± 7.19	85.80 ± 6.71*	84.20 ± 6.28*	87.10 ± 7.11*	89.88 ± 12.35*	7.7 - 101
GGT (U/L)	8.88 ± 2.31	8.60 ± 2.19*	12.10 ± 1.45**	11.88 ± 1.38**	12.40 ± 1.96**	1 - 10
LDH (U/L)	412.50 ± 44.75	387.10 ± 46.84*	486.60 ± 69.19*	397.60 ± 41.62*	430.55 ± 60.04*	83 - 476

*p > 0.05 – statistically significant difference; **p < 0.05 – statistically non-significant difference; sx – standard deviation

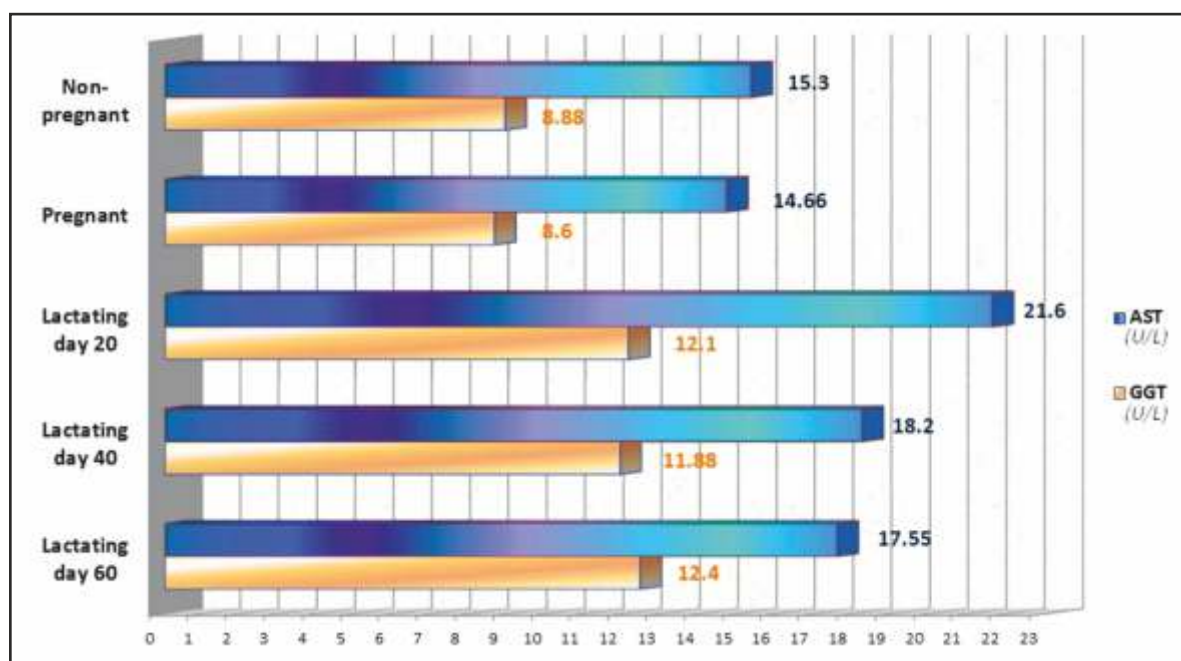


Fig. 2. Dynamics of the mean enzymatic activity of AST and GGT in correlation with the physiological status of the studied group of ewes

ference values for this species. Variations in plasma levels of some biochemical indicators (glucose, cholesterol, triglyceride, and urea) -indicate an energy shortage in early lactating ewes.

In pregnant ewes, lower concentrations of glucose and urea have been determined compared to non-pregnant ewes and higher total protein and albumin concentrations compared to lactating ewes. Increased triglyceride concentrations were recorded in the blood of pregnant ewes compared to non-pregnant ewes.

Mean levels of plasma glucose did not show a statistically significant ($p > 0.05$) decrease in advanced gestation and may be associated with fetal development and maternal glucose mobilization towards the fetal blood flow (15). At the same time, glucose mean values decrease statistically significant ($p < 0.05$) on 20th day of lactation as compared to values obtained in not pregnant ewes. This decrease of the mean glucose values at the onset of lactation can be considered the result of a constant loss of energy through milk produc-

tion. Specifically, these changes suggest that the increased use of glucose for the synthesis of lactose in milk during the early lactation period may be incriminated for the inability to maintain glucose homeostasis.

With the onset of lactation (day 20 and day 40), there was observed a tendency of the mean blood glucose levels to increase, showing a statistically significant increase ($p < 0.05$) as compared to the mean va-

Table 3

The mean values of the biochemical blood profile in correlation with the physiological status of the studied group of ewes ($X \pm sx$)

Parameter	Non-pregnant	Pregnant	Lactating			Reference range
			20 th day	40 th day	60 th day	
Glucose (mg/dL)	90.3 ± 13.03	82.2 ± 11.02*	66.1 ± 8.43**	87.4 ± 12.11*	95.55 ± 14.09*	65 - 118
Urea (mg/dL)	21.00 ± 2.80	19.90 ± 2.59*	23.77 ± 3.11*	24.20 ± 4.01*	25.88 ± 4.08*	10 - 28
Cholesterol (mg/dL)	61.90 ± 8.96	72.20 ± 9.91*	47.90 ± 7.01**	50.33 ± 7.78*	55.50 ± 8.69*	44 - 90
Triglycerides (mg/dL)	10 ± 1.34	15 ± 2.68**	10.5 ± 1.51*	11 ± 2.04*	13 ± 2.55*	-
Creatinine (mg/dL)	0.88 ± 0.09	0.87 ± 0.07*	0.78 ± 0.05*	0.76 ± 0.02*	0.75 ± 0.02*	0.5 - 1.6
Total proteins (g/dL)	6.73 ± 0.46	7.10 ± 0.75*	5.90 ± 0.31*	6.79 ± 0.56*	6.84 ± 0.67*	5.4 - 7.4
Albumin (g/dL)	3.41 ± 0.38	3.69 ± 0.51*	2.80 ± 0.31*	3.28 ± 0.31*	3.31 ± 0.36*	2.7 - 3.7
Total bilirubin (mg/dL)	0.20 ± 0.02	0.32 ± 0.07*	0.41 ± 0.09*	0.27 ± 0.06*	0.25 ± 0.05*	0.1 - 0.6

* $p > 0.05$ - statistically significant difference; ** $p < 0.05$ - statistically non-significant difference; sx - standard deviation

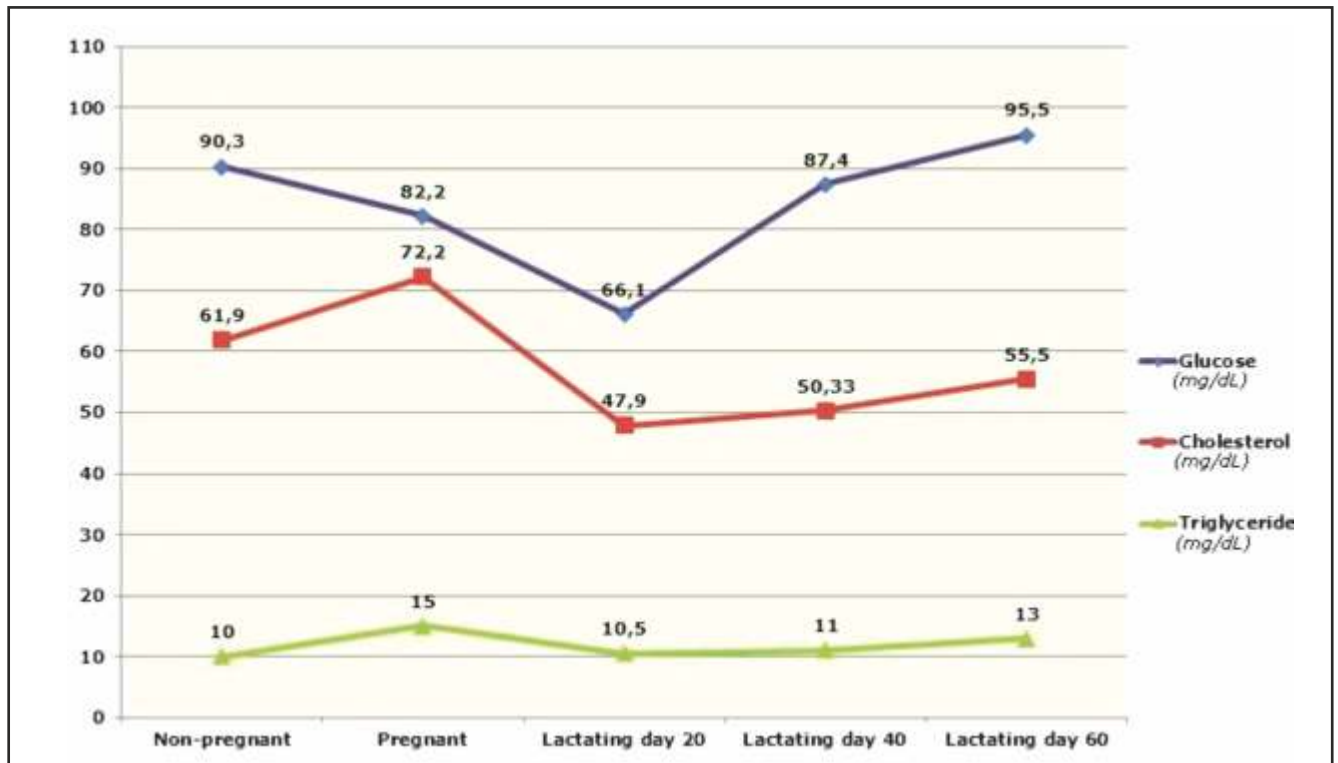


Fig. 3. Dynamics of the mean concentration values of glucose, triglyceride and cholesterol in correlation with the physiological status of the studied group of ewes

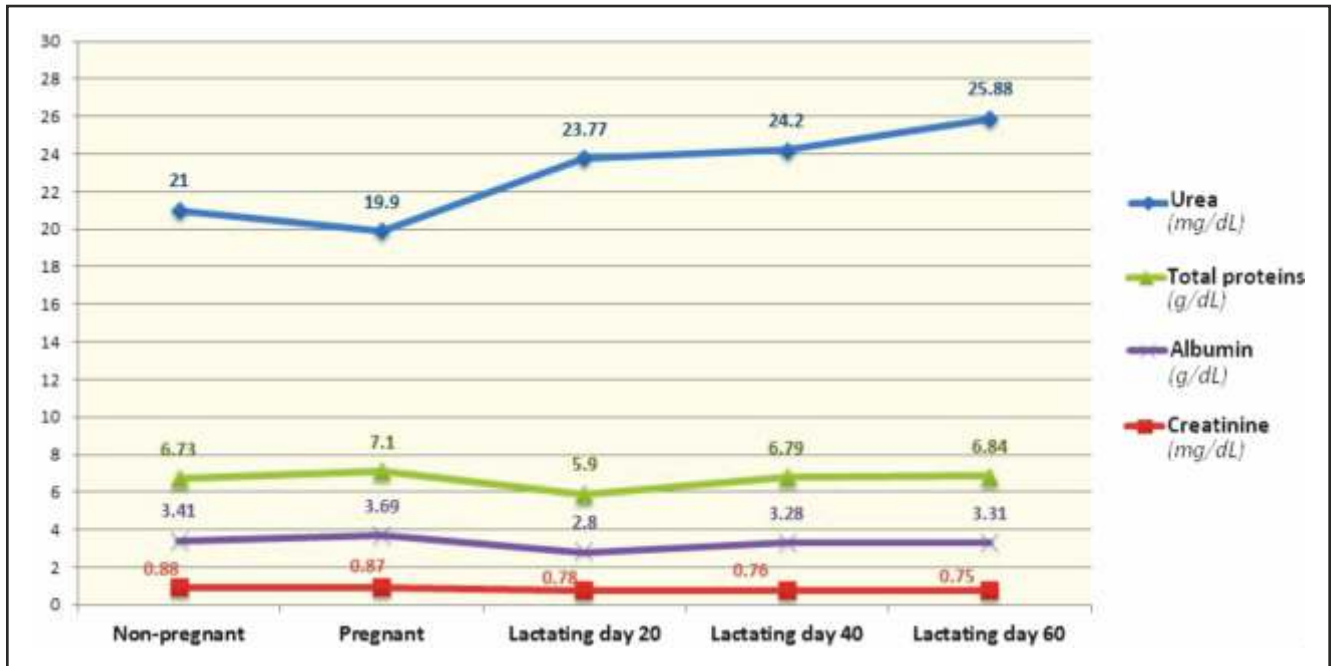


Fig. 4. Dynamics of the mean concentration values of urea, creatinine, total proteins and albumin in correlation with the physiological status of the studied group of ewes

lues of day 20, but no significant differences from the statistical point of view ($p > 0.05$) as compared to the mean values obtained in not pregnant ewes.

A significant increase of the blood glucose levels, but also of the mean concentrations of total proteins, triglycerides and urea as result of the lactation stage progression, was also mentioned by other authors (24). The statistically significant ($p < 0.05$) decrease in glucose concentration at day 20 of lactation may be associated with increased milk production during the incipient lactation period and with an intense activity of the mammary gland. It is well known that in the case of lactating ruminants the tissue requirement of insulin is reduced, which may cause a temporary increase in glucose concentration and thus a stimulation of milk production (25). Elevated urea levels in lactating ewes may be the result of muscle protein catabolism when large amounts of body reserves are mobilized. This aspect comes in direct correlation with the body status score and the body weight of the animal. Caldeira *et al* (2007) mentions that ewes with a lower body status score most likely have higher blood urea concentrations (7).

In pregnant ewes, higher total protein and albumin concentrations were recorded compared to lactating ewes, the differences not being statistically significant ($p > 0.05$). Decreased total protein and albumin concentrations on day 20 of lactation can be explained by the massive mobilization of plasma immu-

noglobulins during the last months of gestation when colostrum is formed in the mammary gland (16).

The mean concentration of triglycerides in pregnant ewes increased significantly. These results can be explained as a reserve of lipoproteins being made for their future massive transport into milk. In early lactation, the mean values of the triglycerides and cholesterol were consistent with the increased energy demand and the negative energy balance of this physiological status. These parameters mean values have been non-significantly lower ($p > 0.05$) compared to not pregnant ewes, but significantly lower ($p < 0.05$) compared to pregnant ewes. The advanced stage of lactation leads to a decrease of the milk production and, consequently, to a reduction in milk fat synthesis (18). The reason behind the increasing triglyceride levels in ewes' blood during lactation can be associated with the negative energy balance that accompanies increased mobilization of fat from the adipose tissue (24).

It was found that along with the progression of lactation, glucose and triglyceride concentrations significantly increased ($p < 0.05$), urea, cholesterol, total protein and albumin concentrations were also slightly increased - but with no statistically significant differences ($p > 0.05$).

There was also recorded a significant increase of the mean plasma total bilirubin concentration in lactating and pregnant ewes compared to the mean values

obtained in not pregnant ewes. This increase may be caused by increased hepatic metabolism in these demanding physiological stages.

Regarding the mean serum creatinine concentrations, the variations are not statistically significant ($p > 0.05$) in correlation with the physiological status nor with the lactation stage.

CONCLUSIONS

Regarding the influence of the physiological status on the blood electrolytic profile in the studied group of ewes, the following conclusions can be made:

Over the course of the study, despite the significant variations observed, the values of the studied electrolytic parameters were generally found in the reference range, with the exception of calcium, whose values decreased significantly ($p < 0.05$) as the milk production intensified and, implicitly, its excretion through milk increased.

Variations in the electrolytic parameters during the study may be associated with the high demand of the fetus, which has an intensive growth rate in advanced gestation and also due to increased milk synthesis during the first third of the lactation period.

Regarding the influence of the physiological status on the blood enzymatic profile in the studied group of ewes, the following conclusions can be made:

The mean blood enzyme activity of the studied group of ewes falls between the reference intervals values for the determined parameters, except for the mean activity of GGT that exceeded the upper limit of the reference range throughout the whole lactation period. The mean enzyme activity of AST showed a significant increase ($p < 0.05$) at the start of the lactation period but did not exceed the upper limit of the reference range. Enhanced enzyme activity may be due to an increased hepatic activity in lactating ewes, an aspect that supports an increased energy and protein demand in order to support milk production.

Regarding the influence of the physiological status on the biochemical blood profile in the studied group of ewes, the following conclusions can be mentioned:

As lactation progresses, glucose and triglyceride concentrations increase significantly ($p < 0.05$), indicating an energy deficiency in early lactating ewes.

The mean glucose levels decrease significantly ($p < 0.05$) on the 20th day of lactation as a result of: energy loss due to the milk synthesis, increased milk production and intense activity of the mammary gland, these changes suggesting that the increased glucose utiliza-

tion for milk lactose synthesis during the early lactation period may be incriminated for the inability to maintain the glucose homeostasis.

Elevated urea levels in lactating ewes may come as a consequence of the muscle protein catabolism when large amounts of body reserves are mobilized.

Decreased total protein and albumin levels on the 20th day of lactation can be explained by massive plasma immunoglobulins mobilization during the last months of gestation when the colostrum is formed in the mammary gland.

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