

# The Changes in the Blood's Acid-Base Balance of the Lacaune Sheep During Different Lactation Stages

Promjena acidobazne ravnoteže krvi u lakon ovaca tijekom različitih stadija laktacije

**Antunović, Z., Mioč, B., Klir Šalavardić, Ž., Širić, I., Držaić, V., Šerić, V., Mandić, S., Novoselec, J.**

Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)

ISSN: 1330-7142 (Print)

<https://doi.org/10.18047/poljo.28.2.8>



Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

# THE CHANGES IN THE BLOOD'S ACID-BASE BALANCE OF THE LACAUNE SHEEP DURING DIFFERENT LACTATION STAGES

Antunović, Z. <sup>(1)</sup>, Mioč, B. <sup>(2)</sup>, Klir Šalavardić, Ž. <sup>(1)</sup>, Širić, I. <sup>(2)</sup>, Držaić, V. <sup>(2)</sup>, Šerić, V. <sup>(3)</sup>, Mandić, S. <sup>(3)</sup>,  
Novoselec, J. <sup>(1)</sup>

Original scientific paper  
Izvorni znanstveni rad

## SUMMARY

**The aim of the present study was to determine the acid-base balance in the blood of the Lacaune sheep during different lactation stages. Thirty lactating Lacaune sheep were involved in the research, and were monitored in the early (day 60), medium (day 120), and late lactation stages (day 180). The following parameters were determined in the blood plasma: pH, partial pressure of carbon dioxide –  $p\text{CO}_2$ , partial pressure of oxygen –  $p\text{O}_2$ , total pressure of carbon dioxide –  $t\text{CO}_2$ , oxygen saturation –  $s\text{O}_2$ , actual base excess  $\text{Cbase} - \text{B}$ , standard base excess  $\text{Cbase} - \text{Ecf}$  and electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  – bicarbonate), while total proteins – TP and albumin ALB were determined in the serum. Also, strong ion difference - SID, z value, anion gap - AG, weak anions, and cations - ATOTtp and ATOTlb, strong ion gap - SIG, unmeasured anions – XA and globulin concentrations were calculated. A significant effect of the lactation stage on most of the acid-base parameters in the blood of the Lacaune sheep was determined, except for  $p\text{CO}_2$ ,  $\text{Cl}^-$ , z-values and the SID. A significant increase of  $p\text{O}_2$ ,  $s\text{O}_2$ , ATOTtp, ATOTlb, AG, SIGalb, SIGtp, XA,  $\text{Na}^+$ , total proteins and albumins, as well as the decreased concentrations of  $\text{K}^+$  and  $\text{Ca}^{2+}$ , were determined in the blood of the Lacaune sheep during lactation. During the medium stage of lactation, significant changes were determined in most of the acid-base parameters in the blood of the Lacaune sheep when compared to the other stages. A lactation stage may significantly affect the acid-base balance parameters in the blood of the Lacaune sheep, although the calculations of SIG, base excess (BE), and unmeasured anion (XA) values should also be included for the obtainment of a more comprehensive picture of the acid-base balance.**

**Keywords:** acid-base balance, blood, lactation stage, Lacaune sheep

## INTRODUCTION

Breeding organizations and breeders are increasingly interested in dairy sheep breeds due to an increased demand for sheep milk and its purchase price on the market. Therefore, the production of sheep milk is increasingly imposed as a significant niche in the improvement of farmers' lives, preventing the depopulation of rural areas. The Lacaune sheep is a French dairy breed which is widely reared and spread in Europe and elsewhere in the world (Barillet et al., 1991). Regarding the parameters of the acid-base balance of ruminants' blood, the previous studies were mostly conducted during pregnancy

or during the transitional period (three weeks prior and subsequent to lambing) (Antunović et al., 2011; Gärtner et al., 2019; Santarosa et al., 2019; Muiño et al., 2021; Zhang et al., 2022), while there is a very small number

(1) Prof. Dr. Zvonko Antunović, Assist. Prof. Željka Klir Šalavardić (zklir@fazos.hr), Assoc. Prof. Josip Novoselec – Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia, (2) Prof. Dr. Boro Mioč, Assist. Prof. Ivan Širić, Assist. Prof. Valentino Držaić – Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia, (3) Assoc. Prof. Vatroslav Šerić, Assist. Prof. Sanja Mandić – Department of Clinical Laboratory Diagnostics, University Hospital Osijek, Jospia Hutlera 4, 31000 Osijek, Croatia

of studies which monitor these parameters during the entire period of lactation (Antunović et al., 2017, 2020). Since the lactation is a demanding period for an animal, it can lead to the deterioration of health, as well as to the changes in parameters of the acid-base balance and oxidative status, so it is necessary to monitor the animal during the entire lactation. The acid–base balance in the blood may indicate the possible metabolic problems, as well as the disorders caused and related to an inadequate feeding (Antunović et al., 2002; Rios et al., 2006). The parameters of the acid-base balance are important in most physiological processes of the lactating animals, for instance in the pulmonary and renal regulatory mechanisms, as well as in the chemical buffer systems (Wagner et al., 2019). As determined by Antunović et al. (2019), more parameters need to be included in the blood acid–base balance, such as a strong ion difference (SID), the calculation of the anion gap (AG), base excess, z values, and the determination of inorganic anions (sulphates, phosphates, etc.) and organic acids (lactate, keto acids). The values of acid–base balance in sheep have not yet been determined for the sheep related to precise negative charge of albumin and globulins, as well as to their dissociation constant, which is still unknown. Gärtner et al. (2019) suggested using more information regarding the acid–base balance in the blood of cows by calculating the weak anion and cation values (ATOTs), SID, strong ion gap (SIGalb), and the unmeasured anions (XA), in comparison to the traditional parameters. For instance, the higher intake of anions (Cl<sup>-</sup>, uremic acids, lactate, unmeasured strong ions and ketoacids) in animals cause acidosis, while a higher cation intake (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) causes alkalosis (Afzaal et al., 2004). Gomez et al. (2020) reported that the SID value is a useful methodology for the quantification of disorders regarding the acid-base balance and for the determination of its development mechanism in goats. In addition,

it is very important to specify the exact values for each sheep breed because the climate zone and region affect the acid–base balance of animals equally.

Therefore, the aim of this research was to determine the acid-base balance in the blood of the Lacaune sheep during different lactation stages.

## MATERIALS AND METHODS

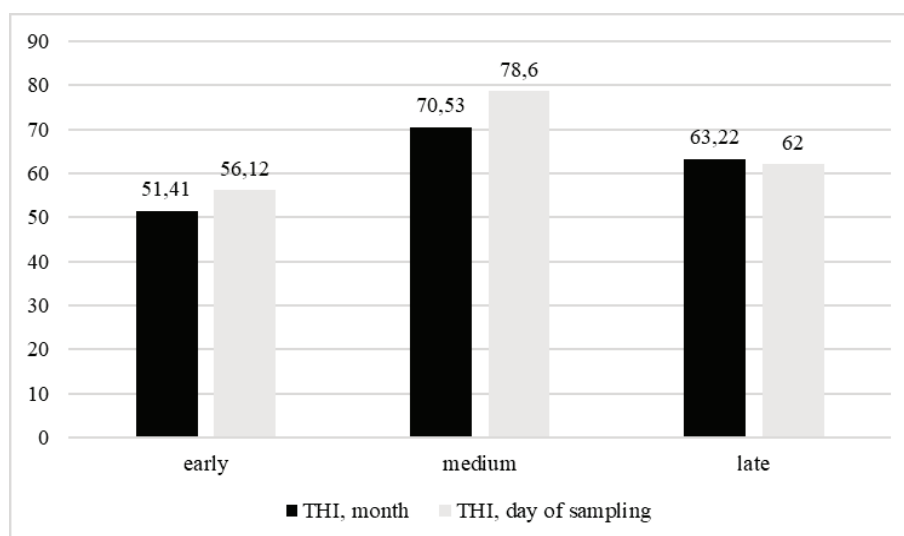
### Experimental design and animal selection

For the research, 30 Lacaune sheep were selected during different stages of lactation from a herd of 200 head at a family farm located in Gundinci (45.15 N, 18.49 E), Republic of Croatia. At the study's onset, the selected ewes were in an early lactation stage (day 60), immediately after the lambs were weaned, and they were re-examined in medium (day 120) and late (day 180) lactation stage. The selected ewes were approximately four years old, with a single lamb in litter, healthy and in a good physical condition. The mean of temperature humidity index (THI) was calculated (Kibler, 1964), based on the measured ambient temperature (Ta; °C) and relative humidity (RH, %) applying the following equation (Eq. (1)):

$$THI = 1.8Ta - (1-RH) \times (Ta - 14.3) + 32 \quad (1)$$

The monthly and daily THI estimated for different lactation stages were 51.41 and 56.12 for the early, 70.53 and 78.60 for the medium, and 63.22 and 62.0 for late stage, respectively (Fig. 1).

The research was carried out by obeying the legal provisions determined by the Animal Protection Act (Republic of Croatia's *Official Gazette* No. 133 (2006), No. 37 (2013), and No. 125 (2013)) and approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek (number of the document 22-04, 5 May 2022).



**Figure 1. Temperature humidity index (THI) during investigation**

Grafikon 1. Temperaturno-vlažni indeks (THI) tijekom istraživanja

### Sheep nutrition

The sheep were fed by the pelleted feed mixture containing 15% of crude protein (minerals: Ca 18876.3 mg/kg; Mg 4639.53 mg/kg; K 17103.2 mg/kg; Na 7042.9 mg/kg; P 10960 mg/kg) in the amount of 1.00 kg/day, a mixture of cereals (1/3 oat and 2/3 barley; minerals: Ca 562.38 mg/kg, Mg 1354.04 mg/kg, K 5154.4 mg/kg, Na 109.27 mg/kg, P 3356 mg/kg) in the amount of 600 g/day and alfalfa hay (13.7% crude proteins; minerals: Ca 6315.28 mg/kg, Mg 2018.09 mg/kg, K 16343.1 mg/kg, Na 1001.3 mg/kg, P 2395 mg/kg) *ad libitum*. They also had animal salt and water *ad libitum*.

### Blood samples and preparation for analyses

Blood samples (10 ml) were taken in the morning from the jugular vein into two sterile vacuum tubes containing Li-heparin. Plasma was analyzed by the automatic Rapid Lab 348 (Siemens Healthcare Diagnostics Inc., USA) analyzer, which works on the basis of ion-selective electrodes. The following parameters were measured: pH value, partial pressure of carbon dioxide -  $p\text{CO}_2$ , partial pressure of oxygen -  $p\text{O}_2$ , total pressure of carbon dioxide -  $t\text{CO}_2$ , oxygen saturation -  $s\text{O}_2$ , actual base excess  $\text{Cbase} - \text{B}$ , standard base excess  $\text{Cbase} - \text{Ecf}$  and electrolytes ( $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  - bicarbonate). According to Stewart (1983), a strong ion difference - SID was calculated while applying Eq. (2). The z-value was calculated as described by Whitehair et al. (1995) following Eq. (3). The anion gap (AG) was calculated following Eq. (4), as described by Kaneko et al. (2008):

$$\text{SID} = [(\text{Na}^+ + \text{K}^+) - \text{Cl}^-] \quad (2)$$

$$\text{z-value} = \text{SID}/\text{Na} \quad (3)$$

$$\text{AG} = [(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)] \quad (4)$$

After that, weak anions and cations (ATOTtp and ATOTalb) were calculated by multiplying the total protein (g/dL) or albumin values by 2.9, as described by Constable (1999) and Waller and Lindinger (2005). According to Gärtner et al. (2019), a strong ion gap (SIG) was calculated, based on the serum concentrations of albumin (ATOTalb, SIGalb), and total proteins (ATOTtp, SIGtp) were computed while applying Eq. (5) and (6). In these equations,  $p\text{Ka}$  was 7.06 (Constable, 2002). The

unmeasured anions (XA) were calculated as described by Gärtner et al. (2019) while applying Eq. (7):

$$\text{SIGalb} = \text{ATOTalb} / (1 + 10^{(p\text{Ka}-\text{pH})}) - \text{AG} \quad (5)$$

$$\text{SIGtp} = \text{ATOTtp} / (1 + 10^{(p\text{Ka}-\text{pH})}) - \text{AG} \quad (6)$$

$$\begin{aligned} \text{XA} = & c\text{Na}^+ + c\text{K}^+ + c\text{Ca}^{2+} + c\text{Mg}^{2+} - c\text{Cl}^- - \\ & c\text{HCO}_3^- - [0.141 \times \text{albumin} \times (\text{pH} - 5.42)] - \\ & [0.04 \times \text{globulin} \times (\text{pH} - 5.58)] - \\ & [\text{phosphate} \times 0.309 \times (\text{pH} - 0.469)] \end{aligned} \quad (7)$$

The ovine blood used for the determination of some blood metabolites was collected in the vacuum tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium). Subsequently, the blood samples were centrifuged at 1609.92 g within 10 minutes after the sampling to obtain the serum samples. The concentrations of total proteins (TP) and albumin (ALB) were determined in the serum on an automatic *Olympus AU 400* device (Olympus, Japan). The globulin content was calculated from the total protein and albumin difference.

### Statistical analyses

The measurement and calculation results were processed by the MEANS procedure, while the influence of the Lacaune sheep lactation stages was analyzed by the GLM procedure and processed by the SAS 9.4®. The means were compared by the Tukey's test, and the differences between the groups of the Lacaune sheep (that is, the stages of lactation) were declared as significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Table 1 presents the effects of the lactation stage on the parameters of the acid-base balance in the blood of the Lacaune dairy sheep. The analysis of Table 1 indicates a significant influence of the stage of lactation on the most of the acid-base parameters in the blood of the Lacaune sheep during lactation, except for the  $p\text{CO}_2$  content. A significant increase in the content of  $p\text{O}_2$  and  $s\text{O}_2$  in the blood was found as lactation progressed to the end. During the medium stage of lactation, significant changes in most of the acid-base parameters in blood were found when compared to the other stages.

**Table 1. Effect of the lactation stage on the parameters of the acid-base balance in the blood of the Lacaune dairy sheep**

Tablica 1. Utjecaj stadija laktacije na pokazatelje acidobazne ravnoteže u krvi lakon ovaca

Parameters / Pokazatelji	Stage of lactation / Stadij laktacije			SEM	P-value / P-vrijednost
	Early / Rani	Medium / Srednji	Late / Kasni		
pH	7.35 <sup>a</sup>	7.30 <sup>b</sup>	7.39 <sup>a</sup>	0.009	<0.001
$p\text{CO}_2$ , kPa	7.80	7.76	7.42	0.100	0.264
$p\text{O}_2$ , kPa	7.52 <sup>b</sup>	10.14 <sup>a</sup>	9.06 <sup>a</sup>	0.224	<0.001
ct $\text{CO}_2$ , mmol/L	26.14 <sup>a</sup>	22.80 <sup>b</sup>	25.70 <sup>a</sup>	0.256	<0.001
C base (B), mmol/L	-2.97 <sup>a</sup>	-7.30 <sup>b</sup>	-3.20 <sup>a</sup>	0.289	<0.001
C base (Ecf), mmol/L	-2.02 <sup>a</sup>	-5.99 <sup>b</sup>	-2.13 <sup>a</sup>	0.276	<0.001
$s\text{O}_2$ , %	72.57 <sup>b</sup>	82.20 <sup>a</sup>	82.18 <sup>a</sup>	1.225	<0.001

SEM-standard error of mean / SEM-standardna pogreška srednje vrijednosti; <sup>a, b</sup> - the values in the rows marked by different letters differ significantly ( $P < 0.05$ ) / <sup>a, b</sup> - vrijednosti u redovima s različitim slovima značajno se razlikuju ( $P < 0,05$ ).

The determined significant changes in most acid-base balance parameters in the blood of the lactating Lacaune sheep are the result of changes that had occurred in the medium stage of lactation when compared to the early and late lactation stages. The deviations found in the parameters of the acid-base balance in the medium stage of lactation might be a result of the extremely high summer temperatures at the time of blood sampling. This probably affected the sheep, which were additionally subjected to stress due to the lactation itself, resulting in a moderate heat stress. Similar changes were found in the calculated parameters of the blood acid-base balance in the Lacaune sheep during lactation, which was expected considering the variations of basic parameters used in the calculations of the blood acid-base balance. Fraser (1991) reported that the pH value of blood is very important in homeostatic priorities. In most enzyme systems, it is influenced by the concentration of  $H^+$  and primarily depends on the bicarbonate and carbonic acid concentration in the blood (Coppock et al., 1982). Although significant changes were found in the blood concerning the pH values of the Lacaune sheep during lactation, they remained within the physiological limits (7.30-7.50), according to Antunović et al. (2010). Sivakumar et al. (2010) found a significant increase in blood pH activity during the heat stress in goats, which contradicts our research. The investigations of two sheep breeds in Oman (Omani sheep and Merino sheep) (Srikandakumar et al., 2002) demonstrated the maintenance of normal acid-base balance during a heat stress by decreasing the blood-related  $pCO_2$ , while  $pO_2$  increased only in the Omani sheep and decreased only in the Merino ones. The authors ascribed this to a higher respiration and oxygen saturation pattern. Sivakumar et al. (2010) determined an increased content of  $O_2$ , C base (B), and c Base (Efc) in the blood of goats exposed to a

longer heat stress. Wojtas et al. (2013) reported that a hyperventilation in the Polish merino sheep resulted in a decreased  $pCO_2$  level and the increased  $CO_2$  and  $pO_2$  levels in blood when the sheep were exposed to a heat stress. In the present research, a similar change was determined in the  $pO_2$  content, indicating that it was induced by a moderate heat-stress level. When the metabolic rate increases, which is normal during lactation, a significant decrease in the blood BE can be expected, leading to a metabolic overload (Castillo et al., 1998). The determined negative BE values in the Lacaune sheep during lactation may be caused due to the fluid balance changes during lactation progress (Castillo et al., 2000). Antunović et al. (2017) determined a decrease of AG and SID, as well as that of  $pO_2$  and  $CO_2$ , in the blood of lactating Croatian spotted goats as the lactation progressed. For instance, Gärtner et al. (2019) concluded that the acid-base balance of blood protein that was deficient in the Holstein cows within one to seventy-six days *post-partum* can be determined much better following a strong ion approach. These authors pointed out that the SID in blood is a suitable indicator for monitoring the acid-base balance in dairy cattle.

In Table 2, the electrolyte concentrations necessary for the acid-base balance calculations in the Lacaune sheep during lactation are presented, as is a significant effect of the lactation stage on the most parameters, except the  $Cl^-$  concentrations. A significant increase of  $Na^+$  concentrations, as well as a significant decrease of  $K^+$  and  $Ca^{2+}$  concentrations, was detected in the blood of the Lacaune sheep as the lactation progressed towards the end. During the medium lactation stage, significant changes in most ovine blood electrolytes compared to other stages, were observed, with an exception of the  $Cl^-$  concentration.

**Table 2. The effect of lactation stage on the blood electrolytes of the Lacaune dairy sheep**

Tablica 2. Utjecaj stadija laktacije na elektrolite u krvi lakon ovaca

Parameters / Pokazatelji	Stage of lactation / Stadij laktacije			SEM	P-value / P-vrijednost
	Early / Rani	Medium / Srednji	Late / Kasni		
Na +	141.00 <sup>c</sup>	146.88 <sup>a</sup>	144.50 <sup>b</sup>	0.350	<0.001
K <sup>+</sup> , mmol/L	5.28 <sup>a</sup>	5.21 <sup>a</sup>	4.66 <sup>b</sup>	0.066	<0.001
Ca <sup>2+</sup> , mmol/L	1.20 <sup>b</sup>	1.27 <sup>a</sup>	1.15 <sup>b</sup>	0.010	<0.001
Cl <sup>-</sup> , mmol/L	110.32	113.48	107.45	1.107	0.096
AG, mmol/L	10.79 <sup>b</sup>	17.58 <sup>a</sup>	14.40 <sup>ab</sup>	1.973	<0.001
HCO <sub>3</sub> <sup>-</sup> , mmol/L	24.35 <sup>a</sup>	21.02 <sup>b</sup>	24.00 <sup>a</sup>	0.246	<0.001

SEM-standard error of mean / SEM-standardna pogreška srednje vrijednosti; <sup>a, b, c</sup> – the values in the rows marked by different letters differ significantly ( $P < 0.05$ ) / <sup>a, b, c</sup> - vrijednosti u redovima s različitim slovima značajno se razlikuju ( $P < 0.05$ ).

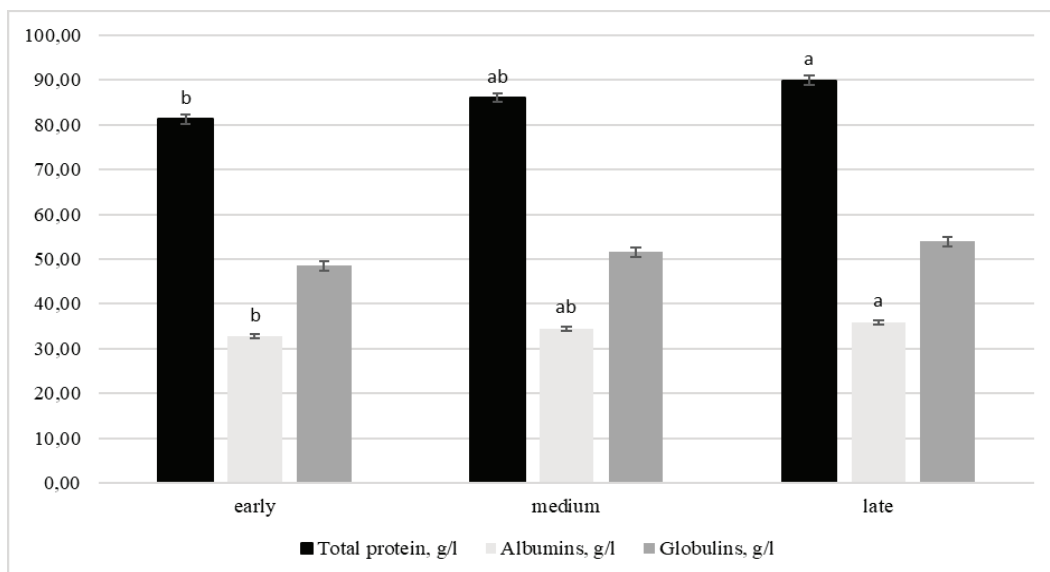
The changes in the blood-related electrolyte concentrations can be ascribed to the alterations in the milk yield of the lactating sheep. The AG is used to demonstrate the presence of unmeasured anions and may be

related to  $pCO_2$  and  $HCO_3^-$  (Fencl and Leith, 1993; Castillo et al., 1998). Yet, the value of AG is limited when assessing the acid-base balance. Ketone bodies, which are the unmeasured anions, were detected while increas-

ing the XA and/or decreasing the SIGalb and SIGtp in the research by Gärtner et al. (2019). The variations of AG in the blood of the Lacaune sheep are connected to the changes in concentrations of total proteins, especially albumins, which were increased in the late stages, resulting in an increased AG, especially in the medium lactation stage. Similar results were reported by Gärtner et al. (2019) concerning the Holstein cows within one to seventy-six days *post-partum*. In the present research, the slightly higher concentrations of Cl<sup>-</sup> in the blood of the lactating Lacaune sheep were measured when compared to the reference values, which are 95-103 mmol/l, while the concentrations of K<sup>+</sup> and Na<sup>+</sup> were within the physiological values (Na= 139-152, K= 3.9-5.4 mmol/L), according to Kaneko et al. (2008). An increase of the Na<sup>+</sup> concentration in the blood of the Lacaune sheep during lactation indicates an elevated activity of the udder with regard to the milk production and lactose synthesis, which implies a free water movement into the udder (Muiño et al., 2021). The opposite trend was recorded for the K<sup>+</sup> concentration, which may be related to an increase in energy provided in the ration and an increased activity of the udder due to the synthesis of different milk components (Muiño et al., 2021). Na<sup>+</sup> is the main extracellular cation, and the Na<sup>+</sup> serum concentration reflects its total body content (Hernandez et al.,

2020). In the study by Castillo et al. (2000), conducted while examining the lactating Murciano-Granadina goats, similar concentrations of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> were found in the high milk producers, as compared to those with a lower milk production. Russel and Russel (2007) pointed out that the hydration of the animals should be known during this assessment. Gärtner et al. (2019) determined an increase of the Na<sup>+</sup> and Cl<sup>-</sup> ions in the plasma of German Holstein cows during the post-partum period, which was probably a result of salt increase in ration, related to the specifics of feeding on the researched farm. Hernandez et al. (2020) reviewed the metabolic profiles in goat and sheep herds and reported that a decrease in the AG also results from an over-hydration, caused by the decreases in the protein concentration and changes in the relative concentration of blood Na<sup>+</sup> and Cl<sup>-</sup>. An increase in the pO<sub>2</sub> and a decrease in HCO<sub>3</sub><sup>-</sup> in the blood of the Lacaune sheep in the medium lactation stage indicates a moderate heat stress. Hernandez et al. (2020) stated that the average concentration of HCO<sub>3</sub><sup>-</sup> in the blood of sheep amounts to 20-25 mmol/l.

The concentrations of total proteins and albumins were significantly increased during lactation, while the concentrations of globulin in the blood of the Lacaune sheep did not significantly differ depending on the lactation stage of lactation (Fig. 2).



**Figure 2. Some blood metabolites of the Lacaune dairy sheep during lactation (<sup>a,b</sup> – the values in the rows marked by different letters differ significantly ( $P < 0.05$ ))**

*Grafikon 2. Neki metaboliti u krvi lakon ovaca tijekom laktacije (<sup>a,b</sup> - vrijednosti u redovima s različitim slovima značajno se razlikuju ( $P < 0,05$ )).*

Table 3 shows the calculated values of acid-base balance parameters in Lacaune sheep which also varied significantly depending on the stage of lactation, except for the z-value and SID. A significant increase in the content of ATOTtp, ATOTalb, AG, SIGalb, SIGtp and XA in the blood of Lacaune sheep during lactation was determined. The determined significant variation of the basic parameters of the acid-base balance in

the medium stage also contributed to significant differences comparing with other stages of lactation. Marai et al. (2004) pointed out that environmental temperature changes significantly affected physiological processes, while heat stress can reduce feed intake, occurrence of irregularities in the metabolism of water, protein, energy and mineral balance as well as changes of blood metabolites concentrations. For determination

of climatic stress on animal THI value is commonly used as an indicator. When THI is 72 and below, it is considered as no heat stress (cool), 73-77 as mild, 78-89 as moderate, and THI above 90 is considered as severe heat stress (Fuquay, 1981). According to Taylor (1992), temperature neutral zone for adult sheep is set among -12 °C and 32 °C (full fleece sheep). Silanikove (1992) reported that the lactating and pregnant ruminants are more prone to the heat stress than the non-lactating and non-pregnant ones. However, Najar et al. (2010) reported that the animals with a high production potential are less tolerant to high temperatures when compared to the animals with a low production potential. Accordingly, in the present research, the Lacaune sheep were exposed to a moderate heat stress (THI = 78.6, Fig. 1) in the medium stage of lactation, since the highest daily temperature amounted to > 35 °C at the time of sampling for five days. In the conditions of heat stress, an accelerated and intensified breathing occurs, which is an important regulatory reaction because it helps an animal to release an excess heat by evaporation from the respiratory system. Therefore, depending

on the animals' length, strength, and exposure to the heat stress, the acid-base balance can be disturbed and deviated from the normal. Thus, the heat stress can cause respiratory alkalosis and compensated metabolic acidosis in cows (Fratric et al., 2010). Compared to other studies in which the animals were exposed to the heat stress for a long period of time, no clear worsening of the indicators of the acid-base blood of the Lacaune sheep was found, which significantly deviates from the physiological values. Nonetheless, in the present study, there was a certain shift in the metabolic processes in a direction of moderate heat-stress effect. The aforementioned fact indicates the good adaptability of the Lacaune sheep. Therefore, it can be concluded that the stage of lactation can significantly affect the parameters of the Lacaune sheep acid-base balance. Therefore, for a proper interpretation of the acid-base balance of the dairy Lacaune sheep during lactation, the calculation of SIG, base excess (BE), and unmeasured anions (XA) values should be considered in order to obtain the most complete picture of the blood-related acid-base balance.

**Table 3. Effect of lactation stage on the calculated parameters of an acid-base balance in the blood of the Lacaune dairy sheep**

Tablica 3. Utjecaj stadija laktacije na izračunate pokazatelje acidobazne ravnoteže u krvi lakon ovaca

Parameters / Pokazatelji	Stage of lactation / Stadij laktacije			SEM	P-value / P-vrijednost
	Early / Rani	Medium / Srednji	Late / Kasni		
ATOT <sub>alb</sub> , mmol/L	20.40 <sup>b</sup>	21.44 <sup>b</sup>	22.34 <sup>a</sup>	0.275	0.014
z values	0.26	0.26	0.27	0.014	0.536
SID, mmol/L	36.18	38.60	38.62	2.013	0.145
SIG <sub>alb</sub> , mmol/L	-2.11 <sup>c</sup>	-1.16 <sup>a</sup>	-1.65 <sup>b</sup>	0.194	<0.001
SIG <sub>tp</sub> , mmol/L	-3.29 <sup>b</sup>	-1.17 <sup>a</sup>	-2.54 <sup>b</sup>	1.168	<0.001
XA, mmol/L	4.69 <sup>c</sup>	11.86 <sup>a</sup>	7.32 <sup>b</sup>	0.531	<0.001

SEM-standard error of mean / SEM-standardna pogreška srednje vrijednosti; <sup>a, b, c</sup> – the values in the rows marked by different letters differ significantly ( $P < 0.05$ ) / <sup>a, b, c</sup> – vrijednosti u redovima s različitim slovima značajno se razlikuju ( $P < 0,05$ ).

## CONCLUSION

The stage of lactation can significantly affect the parameters of the blood acid-base balance of the Lacaune sheep. Significant changes were determined in the most of the acid-base balance parameters in the blood of lactating Lacaune sheep, which are the result of changes that had occurred in the medium stage of lactation if compared to the early and late lactation stages. For a more comprehensive picture, the calculation of SIG, base excess (BE), and unmeasured anions (XA) values should also be included when researching the blood acid-base balance.

## ACKNOWLEDGEMENTS

The study was carried out within the research team Innovative Breeding and Technological Processes in Animal Production (No. 1126) at the Faculty of

Agrobiotechnical Sciences Osijek. The authors wish to thank the farmers from the Orkić family farm who took care of the animals.

## REFERENCES

1. Afzaal, D., Nisa, M., Khan, M. A., & Sarwar, M. (2004). A review on acid base status in dairy cows: implications of dietary cation-anion balance. *Pakistan Vet J*, 24(4), 199-202.
2. Antunović, Z., Senčić, Đ., Šperanda, M., & Liker, B. (2002). Influence of the season and the reproductive status of ewes on blood parameters. *Small Ruminant Research*, 45(1), 39-44. [https://doi.org/10.1016/S0921-4488\(02\)00109-8](https://doi.org/10.1016/S0921-4488(02)00109-8).
3. Antunović, Z., Šperanda, M., Novoselec, J., & Đidara, M. (2010). Nutrition of lambs and acid-base balance. *Krmiva* 52(6), 333-338. (in Croatian).

4. Antunović, Z., Novoselec, J., Šperanda, M., Đidara, M., Klir, Ž., & Kopačin, T. (2011). Blood acid-base balance and hematological parameters in pregnant goats from organic farming. *Krmiva*, 53(4), 151-153. (in Croatian).
5. Antunović, Z., Šperanda, M., Novoselec, J., Đidara, M., Mioč, B., Klir, Ž., & Samac, D. (2017). Blood metabolic profile and acid-base balance of dairy goats and their kids during lactation. *Veterinarski arhiv*, 87(1), 43-55.
6. Antunović, Z., Marić, I., Šerić, V., Mioč, B., & Novoselec J. (2019). Haemato-biochemical profile and acid-base status of Croatian spotted goats of different ages. *Archives Animal Breeding*, 62(2), 455-463. <https://doi.org/10.5194/aab-62-455-2019>
7. Antunović, Z., Novaković, K., Klir, Ž., Šerić, V., Mioč, B., Ronta, M., & Novoselec, J. (2020). Blood metabolic profile and acid-base status of Istrian goats. *Veterinarski arhiv*, 90(1), 27-38. <https://doi.org/10.24099/vet.arhiv.0780>.
8. Barillet, F., Marie, C., Jacquin, M., Lagriffoul, G., & Astruc, J. M. (2001). The French Lacaune dairy sheep breed: use in France and abroad in the last 40 years. *Livestock Production Science*, 71(1), 17-29. [https://doi.org/10.1016/S0301-6226\(01\)00237-8](https://doi.org/10.1016/S0301-6226(01)00237-8)
9. Castillo, C., Hernandez, J., Miranda, M., Lopez, M., Ayala, I., & Benedito, J. L. (1998). Analysis of acid-base balance by application of Fencel's equations in sheep. *Journal of Applied Animal Research*, 14, 127-135. <https://doi.org/10.1080/09712119.1998.9706691>.
10. Castillo, C., Hernandez J., Benedito, J., Lopez-Alonso, M., Miranda C., Gutierrez-Panizo, C., & Sotillo, J. (2000). Quantitative evaluation of acid-base balance on milk producing goats: Effect of sex and milk yield. *Veterinarní medicína*, 45, 241-246.
11. Constable, P. D. (1999). Clinical assessment of acid-base status: strong ion difference theory. *Veterinary Clinics of North America: Food Animal Practice*, 15(3), 447-471. [https://doi.org/10.1016/S0749-0720\(15\)30158-4](https://doi.org/10.1016/S0749-0720(15)30158-4)
12. Constable, P. D. (2002). Calculation of variables describing plasma non-volatile weak acids for use in the strong ion approach to acid-base balance in cattle. *American Journal of Veterinary Research*, 63, 482-90. <https://doi.org/10.2460/ajvr.2002.63.482>
13. Coppock, C. E., Grant, P. A., Portzer, S. J., Charles, D. A., & Escobosa, A. (1982). Lactating dairy cow responses to dietary sodium, chloride and bicarbonate during hot weather. *Journal of Dairy Science*, 65, 566. [https://doi.org/10.3168/jds.S0022-0302\(82\)82234-0](https://doi.org/10.3168/jds.S0022-0302(82)82234-0)
14. Fencel, V., & Leith, V. E. (1993). Frontiers in respiratory physiology: Stewart's quantitative acid-base chemistry. *Respiration Physiology*, 91, 1-16. [https://doi.org/10.1016/0034-5687\(93\)90085](https://doi.org/10.1016/0034-5687(93)90085)
15. Finocchiaro, R., van Kaam, J. B. C. H. M., Portolano, B. & Misztal, I. (2005). Effect of heat stress on production of Mediterranean dairy sheep. *Journal of Dairy Science*, 88, 1855-1864. [https://doi.org/10.3168/jds.S0022-0302\(05\)72860-5](https://doi.org/10.3168/jds.S0022-0302(05)72860-5)
16. Fraser, C. M. (1991). Acid-base balance. In: The Merck Veterinary Manual, 7th ed. Merck&Co., Inc., Rahway, NJ, pp. 1361-1367.
17. Fratrić, N., Vujanac, I., Šamanc, H., Kirovska, D., Gvozdić, D., & Adamović, M. (2010). Determination of NABE in urine and high-yield dairy cows in early lactation in conditions of moderate heat stress. *Veterinarski glasnik*, 64(5-6), 349-358.
18. Fuquay, J. W. (1981). Heat stress as it affects animal production. *Journal of Animal Science*, 52, 164-169. <https://doi.org/10.2527/jas1981.521164x>
19. Gomez, D. E., Bedford, S., Darby, S., Palmisano, M., MacKay, R. J., & Renaud, D. L. (2020). Acid-base disorders in sick goats and their association with mortality: A simplified strong ion difference approach. *Journal of Veterinary Internal Medicine*, 34, 2776-2786. <https://doi.org/10.1111/jvim.15956>
20. Gärtner, T., Zoche-Golob, V., Redlberger, S., Reinhold, P., & Donat, K. (2019). Acid-base assessment of post-parturient German Holstein dairy cows from jugular venous blood and urine: A comparison of the strong ion approach and traditional blood gas analysis. *PLOS one*, 14, e0210948. <https://doi.org/10.1371/journal.pone.0210948>
21. Hernandez, J., Benedito, L., & Castillo, C. (2020). Relevance of the study of metabolic profiles in sheep and goat flock. Present and future: A review. *Spanish Journal of Agricultural Research*, 18(3), e06R01. <https://doi.org/10.5424/sjar/2020183-14627>
22. Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (2008). Clinical biochemistry of domestic animals. 6th ed. Elsevier/Academic Press, Amsterdam. pp. 931.
23. Kibler, H. H. (1964). Thermal effects of various temperature-humidity combinations on Holstein cattle as measured by eight physiological responses. *Research Bulletin Missouri Agricultural Experiment Station*, 862, 1-42.
24. Marai, I. F. M. (2004). Reproductive traits and the physiological background of the seasonal variations in Egyptian Suffolk ewes under the conditions of Egypt. *Annals of Arid Zone*, 42, 1-9.
25. Muiño, R., Hernández, J., Benedito, J. L., & Castillo, C. (2021). Effects of calving body condition score on blood acid-base balance of primiparous Holstein-Friesian dairy cows in a commercial dairy farm: a case study. *Animals*, 11, 2075. <https://doi.org/10.3390/ani11072075>
26. Najar, T., Rejeb, M., & Ben M. Rad, M. (2010). Modelling the effects of heat stress on some behavior and physiological parameters in cows. In: D. Sauvant, J. Van Milgen, P. Faverdin, N. Friggens (eds), Modelling Nutrient Digestion and Utilization in Farm Animals. Wageningen Academic Publishers, The Netherlands, pp. 130-136.
27. Rios, C., Marin, M. C., Catafau, M., & Wittwer, F. (2006). Relationship between blood metabolism ( $\beta$ -hydroxybutyrate, NEFA, cholesterol and urea) and nutrition balance in three goat herds under confinement. *Archivos de Medicina Veterinaria*, 38, 19-23.
28. Russell, K. E., & Roussel, A. J. (2007). Evaluation of the ruminant serum chemistry profile. *Veterinary Clinics of North America: Food Animal Practice*, 23(3), 403-426. <https://doi.org/10.1016/j.cvfa.2007.07.003>
29. Santarosa, B. P., Dantas, G. N., Ferreira, D. O. L., Carvalho, M. G., Rodrigues, M., Pereira, P. F. V., Silva, A. A., & Gonçalves, R. C. (2019). Comparison of electrolyte and acid-base balances of Dorper breed ewes between



- single and twin pregnancies. *Pesquisa Veterinaria Brasileira*, 39(10), 789-795.  
<https://doi.org/10.1590/1678-5150-PVB-5952>
30. SAS 9.4® (2002-2012) SAS Institute Inc., Cary, NC, USA
31. Silanikove, N. (1992). Effects of water scarcity and hot environment on appetite and digestion in ruminants: a review. *Livestock Production Science*, 30, 175-194.
32. Sivakumar, A. V. N., Singh, G., & Varshney, V. P. (2010). Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Australasian Journal of Animal Sciences*, 23(11), 1462-1468.  
<https://doi.org/10.5713/ajas.2010.90471>
33. Srikandakumar, A., Johnson, E. H., & Mahgoub, O. (2003). Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Ruminant Research*, 49, 193-198.  
[https://doi.org/10.1016/S0921-4488\(03\)00097-X](https://doi.org/10.1016/S0921-4488(03)00097-X)
34. Stewart, P. A. (1983). Modern quantitative acid-base chemistry. *Canadian Journal of Physiology and Pharmacology*, 61, 1441-1461.  
<https://doi.org/10.1139/y83-207>
35. Wagner C. A., Silva P. H. I., & Bourgeois S. (2019). Molecular pathophysiology of acid-base disorders. *Seminars in Nephrology*, 39(4), 340-352.  
<https://doi.org/10.1016/j.semnephrol.2019.04.004>
36. Taylor, R. E. (1992). Adaptation to the environment. In: *Scientific Farm Animal Production*, Macmillan Publishing Company, New York, NY, pp. 326-332.
37. Waller, A., & Lindinger, M. I. (2005). Physicochemical analysis of acid-base status during recovery from high-intensity exercise in Standardbred racehorses. *Equine and Comparative Exercise Physiology*, 2(2), 119-127. <https://doi.org/10.1079/ECP200549>
38. Whitehair, K. A., Haskins, S. C., Whitehair, J. G., & Pascoe, P. J. (1995). Clinical applications of quantitative acid-base chemistry. *Journal of Veterinary Internal Medicine*, 9, 1-11.  
<https://doi.org/10.1111/j.1939-1676.1995.tb03265.x>
39. Wojtas, K., Cwynar, P., Kolacz, R., & Kupczynski, R. (2013). Effect of heat stress on acid-base balance in Polish Merino sheep. *Archiv Tierzucht*, 56, 92, 917-923.  
<https://doi.org/10.7482/0003-9438-56-092>
40. Zhang, X., Glosson, K. M., Bascom, S. S., Rowson, A. D., Wang, Z., & Drackley, J. K. (2022). Metabolic and blood acid-base responses to parturition dietary cation-anion difference and calcium content in transition dairy cows. *Journal of Dairy Science*, 105.  
<https://doi.org/10.3168/jds.2021-21191>

## PROMJENA ACIDOBAZNE RAVNOTEŽE KRVI U LAKON OVACA TIJEKOM RAZLIČITIH STADIJA LAKTACIJE

### SAŽETAK

**Cilj rada bio je utvrditi acidobaznu ravnotežu u krvi lakon ovaca tijekom različitih stadija laktacije. Za istraživanje je odabrano 30 lakon ovaca u laktaciji, koje su praćene u ranome (60. dan), srednjem (120. dan) i kasnom stadiju laktacije (180. dan). U krvnoj plazmi utvrđeni su pH, parcijalni tlak ugljičnoga dioksida ( $p\text{CO}_2$ ), parcijalni tlak kisika ( $p\text{O}_2$ ), ukupni tlak ugljičnoga dioksida ( $t\text{CO}_2$ ), saturacija kisikom ( $s\text{O}_2$ ), stvarni i standardni višak baza ( $Ea$  i  $Ecf$ ) i elektroliti ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  i  $\text{HCO}_3^-$  – bikarbonati), a u serumu ukupni proteini (TP) i albumini (ALB). Također su izračunane sljedeće vrijednosti: razlika jakih iona (SID), z-vrijednost, anionski procjep (AG), slabi anioni i kationi (ATOT<sub>tp</sub> and ATOT<sub>alb</sub>), jaki ionski procjep (SIG), nemjereni anioni (XA) i koncentracije globulina. Utvrđen je značajan utjecaj stadija laktacije na većinu pokazatelja acidobazne ravnoteže u krvi lakon ovaca, osim sadržaja  $p\text{CO}_2$ ,  $\text{Cl}^-$ , z-vrijednosti i SID-a. Utvrđeno je značajno povećanje sadržaja  $p\text{O}_2$ ,  $s\text{O}_2$ , ATOT<sub>tp</sub>, ATOT<sub>alb</sub>, AG, SIG<sub>alb</sub>, SIG<sub>tp</sub>, XA,  $\text{Na}^+$ , ukupnih proteina i albumina te smanjenje koncentracija  $\text{K}^+$  i  $\text{Ca}^{2+}$  u krvi lakon ovaca kako je laktacija odmicala kraju. Tijekom srednjega stadija laktacije utvrđene su značajne promjene većine pokazatelja acidobazne ravnoteže krvi lakon ovce u odnosu na druge stadije. Stadij laktacije može značajno utjecati na pokazatelje acidobazne ravnoteže krvi lakon ovaca te bi za potpuniju sliku pri izradi acidobazne ravnoteže trebalo uključiti i izračun SIG-a, viška baza (BE) i nemjerenih aniona (XA).**

**Ključne riječi:** acidobazna ravnoteža, krv, stadij laktacije, lakon pasmina ovaca

(Received on September 13, 2022; accepted on November 9, 2022 – Primljeno 13. rujna 2022.; prihvaćeno 9. studenoga 2022.)