

**Correlation between pre and post-slaughter physiological parameters in lambs**

**Correlação entre parâmetros fisiológicos pré e pós-abate em cordeiros**

**Correlación entre los parámetros fisiológicos previos y posteriores al sacrificio en por de corderos**

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## Resumo

O objetivo deste estudo foi obter informações com validade científica a respeito das prováveis alterações fisiológicas que os ovinos podem sofrer no período antecedente ao abate e sua relação com condições de bem-estar e manutenção da homeostase. Foram utilizados 29 cordeiros machos castrados, clinicamente saudáveis, sem raça definida e com idades até um ano. Previamente ao abate, foram aferidas as frequências cardíaca e respiratória, assim como a temperatura retal. No momento da sangria foi coletada amostra de sangue para a realização da análise dos parâmetros hematológicos (ácido láctico, glicose, hematócrito). O valor médio encontrado para a temperatura retal foi de  $39,30 \pm 0,45$  °C, para frequência respiratória de  $64,10 \pm 28,52$ mov/min, frequência cardíaca de  $106,90 \pm 30,57$ bat/min, ácido láctico de  $4,81 \pm 1,04$ mmol/L, glicose de  $69,79 \pm 10,33$ mg/dL e hematócrito de  $38,93 \pm 3,28\%$ . Através da análise dos dados verificou-se correlação baixa e positiva entre a frequência respiratória e temperatura retal. Conclui-se que cordeiros mantidos sob temperaturas elevadas demonstraram um aumento nas frequências respiratória e cardíacas, bem como intensificação da condição de estresse refletida pelo incremento nos níveis de ácido láctico plasmático.

**Palavras-chave:** Ovinos; Estresse; Ácido láctico; Frequência cardíaca; Frequência respiratória; Temperatura corporal.

## Abstract

The objective of this study was to obtain information with scientific validity regarding the probable physiological alterations that sheep may suffer in the period prior to slaughter and its relationship with welfare conditions and maintenance of homeostasis. Twenty-nine castrated male lambs were used, clinically healthy, with no defined breed, and aged up to one year. Before slaughter, the heart and respiratory frequencies were measured, as well as the rectal temperature. At the time of bleeding, a blood sample was collected for analysis of hematological parameters (lactic acid, glucose, hematocrit). The mean value found for the rectal temperature was  $39.30 \pm 0.45$  °C, for respiratory rate of  $64.10 \pm 28.52$ mov/min, heart rate of  $106.90 \pm 30.57$ bat/min, lactic acid of  $4.81 \pm 1.04$ mmol/L, glucose of  $69.79 \pm 10.33$ mg/dL and hematocrit of  $38.93 \pm 3.28\%$ . The database analysis showed a low and positive correlation between respiratory rate and rectal temperature. It was concluded that lambs kept at high temperatures showed an increase in respiratory and cardiac frequencies, as well as an intensification of the stress condition reflected by the increase in plasma lactic acid levels.

**Keywords:** Sheep; Stress; Lactic acid; Heart rate; Respiratory rate; Body temperature.

## Resumen

El objetivo del estudio fue obtener información con comprobación científica acerca de los probables cambios fisiológicos que puede sufrir el ovino en el período previo al sacrificio y su relación con las condiciones de bienestar y mantenimiento de la homeostasis. Se utilizaron 29 corderos machos castrados, clínicamente sanos, mestizos y de hasta un año de edad. Antes del sacrificio, se midieron las frecuencias cardíaca y respiratoria, así como la temperatura rectal. En el momento del sangrado se tomó una muestra de sangre para realizar el análisis de parámetros hematológicos (ácido láctico, glucosa, hematocrito). El valor medio encontrado para la temperatura rectal fue de  $39.30 \pm 0.45$  °C, para una frecuencia respiratoria de  $64.10 \pm 28.52$  mov/min, frecuencia cardíaca de  $106.90 \pm 30.57$  bat/min, ácido láctico de  $4,81 \pm 1,04$  mmol / L, glucosa de  $69,79 \pm 10,33$  mg / dL y hematocrito de  $38,93 \pm 3,28\%$ . El análisis de datos mostró una correlación baja y positiva entre la frecuencia respiratoria y la temperatura rectal. Se concluye que los corderos mantenidos a altas temperaturas mostraron un aumento de la frecuencia respiratoria y cardíaca, así como una intensificación de la condición de estrés reflejada por el aumento de los niveles plasmáticos de ácido láctico.

**Palabras clave:** Oveja; Estrés; Ácido láctico; Frecuencia cardíaca; Frecuencia respiratoria; Temperatura corporal.

## 1. Introduction

Sheep farming is an activity practiced in most countries of the world, demonstrating the easy adjustment of the sheep species (*Ovis aries*) to different climates and environmental conditions. Currently, most sheep farms are focused on meat production, with the most diverse systems (Sorio, Magalhães & Marques, 2016). However, the development of the consumer market still contrasts with the difficulties of interaction of the links of the productive chain of cut ovine culture, which implies, mainly, in the disorganization of the different sectors involved in Brazil (Guimarães & Souza, 2014). Allied to this, consumers are increasingly demanding, looking for products with better organoleptic characteristics, being relevant aspects, from the breeding of animals to the processing of carcasses (Sorio et al., 2016).

In addition, in order to obtain meats with superior quality standards, issues such as well-being should be taken into account (Lima, 2014), changes in the environment the animal is used to may cause stress, inducing the use of physiological mechanisms to restore

homeostasis and promote adaptation (Hemsworth, Rice, Edwards, Ponnampalam & Coleman, 2016).

In this context, it becomes essential to seek information about factors that may affect the quality of the product. Pre-slaughter stress is one of these factors, where the stress reactions represent modifications of the physiological responses that have as objective the maintenance of the animal's homeostasis (Paes, Gonçalves, Barioni, Leme, Melo & Cruz, 2012). However, analyses on the influence of stress on meat quality are high cost or difficult and complex to measure on the property and/or slaughterhouse (Ramos, 2012; Lima, 2014).

Variables such as lactic acid levels and glycemia are directly associated with the physiological condition of the animals in the pre-slaughter and influence the final quality of the meat (Issakowicz, 2016). The muscle pH decline curve, due to lactic acid accumulation, is one of the most significant biochemical changes during muscle to meat conversion (Rodrigues & Silva, 2016). If the acidification effect does not achieve adequate levels of defects, such as PSE (Pale, Soft, Exudative) and DFD (Dark, Firm, Dry) type meats may occur (Hemsworth et al., 2016).

Stempa, Muchenje, Abrahams & Bradley (2016) find the influence of stressing causes in the quality characteristics of the final product evaluated through post-slaughter parameters in animals of different categories, breeds, and sex of sheep. Variables such as respiratory rate (Silanikove, 2000), lactic acid levels (Maiorano, Macedo, Generoso, Curi, Chardulo, Castilhos, Silva, 2016; Miranda-de lama, Rivero, Chacón, Garcia-Belenguer, Villarroel, Maria, 2010), and glycemia are used as indicators of physiological stress (Lima, 2014). These variables can quantify the degree of stress in ruminants since animals submitted to discomfort due to pre-slaughter management will have changes in these parameters (Cottrell, McDonagh, Dunseha & Warner, 2008; Lima, 2014; Silva et al., 2017).

As an increase in the primary sources of energy generation at blood level, especially glucose, which in aerobic conditions will have carbon dioxide and water as the final product, but when this route is compromised, that is, when there is a lack of oxygen, the anaerobic route will be used obtaining lactic acid as the final product. Also, the first variables to present a difference are the cardiac and respiratory frequencies, since in the first phase of stress, called alarm response, they are the main parameters that suffer alterations, where these variables increase to prepare the organism for this new situation.

In view of the above, this study aimed to assess physiological variables in pre-killing and post-killing hematological parameters of clinically healthy lambs sacrificed under humanitarian slaughter conditions, in order to establish the likely correlations between them.

## 2. Methodology

The study was developed with the approval of the Ethics Committee on Animal Experimentation of the Federal University of Pelotas (Protocol No. 47161-2018).

The collection of samples was carried out at the frigorific company with sanitary inspection, located in the city of São Lourenço do Sul - RS, on January 29, 2019, obeying the technical standards for humanitarian slaughter established by the Division of Inspection of Products of Animal Origin (DIPOA), agency of the Secretariat of Agriculture, Livestock and Rural Development of the State of Rio Grande do Sul, and thus inspected by the State Inspection Service. However, the establishment has adhered to the Brazilian Inspection System (SISBI), which enables the marketing of its products throughout the country.

The animals used were castrated males (n=29) of the ovine species, approximate age of eight months with no defined breed (SRD) and coming from a confinement located in the city of São Lourenço do Sul (31° 21' 46 "S : 51° 58' 44 "W), Rio Grande do Sul, Brazil.

The transport to the frigorific took place by truck, in a 20km journey, in approximately 35 minutes, under a capacity of 0.29m<sup>2</sup>/ lamb. Before slaughter, the animals were submitted to food fasting for 20h, obeying the rules described by Ordinance No. 62 (Government of Brazil, 2018), remaining in the waiting pens with water at will and density of 2.5m<sup>2</sup>/animal.

On the day of slaughter, moments before entering the stun box, the animals were manually contained by a trained worker to check the following physiological parameters: rectal temperature (RT), heart rate (HR), and respiratory rate (RR). The ambient temperature was 27.4 °C. The lambs were individually identified with a numbered plastic plate, which was attached to the distal end of the right forearm with a nylon clamp. It is important to emphasize that although the animals were physically contained for the collection of the parameters, it can be assumed that it did not influence the variables evaluated, since the containment did not exceed one minute (Stilwell, Carvalho, Lima & Broom, 2008).

The RR, in movements per minute (mov/min), was measured by observing the movements of the animals' left flank. The HR, in beats per minute (bat/min), was obtained by auscultation between the third and fourth intercostal space, near the costochondral joint, in the animal's left flank. Both RR and HR were evaluated for 15 seconds with the aid of a stopwatch and multiplying the value by four to determine the number of movements or beats per minute. The TR (°C) was checked by means of a digital thermometer kept on the rectum of the animal until the sounder shot.

Later, the animals were insensitized by means of a pneumatic penetrative dart gun, with pressure from 190 to 200 psi, and immediately hoisted to section the jugular veins and carotid arteries for bleeding. In the bleeding, 5 mL of blood was collected in tubes with an aqueous solution of heparin sodium. After collection, the blood samples were homogenized by gentle manual inversion 5 to 10 times (Andriolo, Cançado, Barbosa, Vieira, Mendes, Sumita, Romano, Castro, Oliveira, 2010). The blood collected was used for the following hematological measurements: measurement of hematocrit, blood glucose and lactic acid levels.

Blood glucose (mg/dL) was measured with a portable glucometer (Acuu-Chek Performa®); lactic acid (mmol/L) was measured with a portable lactometer (Accutrend Plus®). The hematocrit values were obtained by the microhematocrit technique in capillary tubes and centrifugation, at 10.000 rpm per 10min.

Statistical analysis was performed by means of linear correlation analysis (Pearson) between the variables studied, in order to establish the degree of association between them by accepting a 10% confidence limit. The assumption of normality of the data was tested by Kolmogorov-Smirnov and Lilliefors tests (StatSoft, Tulsa, OK, USA).

### **3. Results and Discussion**

A widely used parameter to determine the degree of adaptability of the animals is the TR, since it is a good indicator of internal body temperature (Lima, 2014). According to Reece (2017a) sheep are well adapted to both cold and heat, and have an average body temperature of 39.1°C, with a physiological variation of 38.3°C to 39.9°C, where values outside this range may indicate some abnormality.

According to Cezar, Souza, Souza, Filho, Tavares & Medeiros (2004), the TR of the sheep suffers oscillations during the day, with lower values being noticed during the morning in relation to the afternoon. This information has relevant practical implications for understanding the mechanisms used by sheep to reach equilibrium.

However, in the experiment carried out by Starling, Silva, Cerón-Muñoz, Barbosa & Costa (2002) no variation was found in the TR of Corriedale sheep at different temperatures (20°C, 30°C and 40°C), which were above the confidence interval of this work. The authors associated the results obtained with the efficiency of evaporative thermolysis in the process of maintaining homeothermia.

When there are oscillations in environmental variables the animal organism seeks balance through physiological changes, which can be observed in parameters such as RR and HR (Luz, Fonseca, Junior, Souza, Amorim, Silva, Lima, Junior & Santos, 2014).

RR can be used to quantify the degree of stress in ruminants. According to Reece (2017b) sheep in season, ruminating, with fur from 0.5 to 3.6cm, at 18°C, RR is 20 to 34 movements per minute (mov/min), with an average of 25mov/min, and the data observed in this work were within a range between 60-80 mov/min, characterizing a high average stress (Silanikove, 2000).

The HR of resting sheep is around 70 to 80 beats per minute (bat/min), and may be influenced by some excitement, such as at the time of containment of animals (Kline, Hasser & Heesch, 2017). The values found in this work were above the reference values.

However, the values for RR and HR corroborate with those reported by Correa, Cardoso, Castanheira, Landim, Dallago, Louvandini & McManus (2012), who found an average for RR of 64/miv/min and for HR of 118 bat/min, also higher than the reference data, when they evaluated pure-bred crossed and delanded animals, where sheep cross Texel x Santa Inês presented the highest values in both variables.

Amaral, Barbosa, Gasparino, Akimoto, Lourenço & Santello (2009) observed in woolen sheep RR of 109.8 and 80.7 mov/min, for the breeds Texel and Ile de France, respectively. Values higher than those found in this work, under similar temperatures, around 27°C. For HR, the analyzed levels were lower, with 89.67 bat/min for Texel and 89.63 bat/min for Ile de France. Starling et al. (2002) when they evaluated the tolerance of Corriedale sheep to thermal stress, found higher values of RR than in this work, being that for environmental temperature of 20°C the RR found was 124.9 mov/min and 161.3 mov/min at room temperature of 30°C, showing the susceptibility of the breed to temperature oscillations. The values obtained by these authors can be explained by the need to increase blood flow to the body surface by seeking thermoregulation by heat dissipation (Silanikove, 2000).

In the present study, in face of the condition of high environmental temperature (27.4°C) one can clearly perceive the compensation in the heat dissipation for the high heart and respiratory frequencies and above the reference ranges cited in the literature, which possibly was responsible for the maintenance of the body temperature within the physiological values for the sheep species.

The pre-slaughter parameters presented TR values within the reference standards, whereas the HR and RR variable levels were above, data shown in Table 1.

**Table 1.** Mean, standard deviation (SD) and confidence interval (95% CI) of rectal temperature (°C), heart rate (bat/min) and respiratory rate (mov/min) of lambs without defined race (SRD) at pre-slaughter (n=29).

Variable	Average ± SD	IC 95%	Reference values*
Rectal temperature (°C)	39.30 ± 0.45	39.14 – 39.47	38.3 – 39.9
Heart rate (bat/min)	106.90 ± 30.57	95.77 – 118.02	70 – 80
Respiratory rate (mov/min)	64.14 ± 28.52	53.76 – 74.52	20 – 34

\*REECE, (2017). Source: Authors.

Ross & Kitis (1969) proved that the change in lactic acid levels in the blood is immediate after some excitement and can increase the resting level about twice, and a reference range for healthy sheep is 1.00-1.33 mmol/L (Kaneko, Harvey & Brass, 2008).

Lactic acid levels can be used to indicate the occurrence of stress in ruminants (Maiorano et al., 2016; Miranda-de la lama et al., 2010). Teke, Ekiz, Akdag, Ugurlu, Gulay & Senturk (2014) observed the effects of different densities on transport (0.20 and 0.27 m<sup>2</sup>/lamb), reporting significantly higher values for lactic acid levels in lambs under higher density after transport (3.27 mmol/L versus 2.82 mmol/L). In contrast, Cozar, Rodriguez, Garijo, Calvo & Vergara (2016) found no significant variations of this parameter in Merino lambs transported in densities between 0.16-0.30 m<sup>2</sup>/animal (2.55 mmol/L and 2.22 mmol/L, respectively). In the present study, the observed values (4.81 mmol/L) were above those verified by these authors.

Stempa et al. (2016) verified the effects of sex and race (Merino and Dorper) on plasma lactate levels, indicating that females have higher levels compared to males (7.60 mmol/L and 4.87 mmol/L, respectively). In addition, the Dorper breed produced higher levels of plasma lactate than Merino (7.74 mmol/L and 4.73 mmol/L, respectively). In this study, the values found were similar to those described for the Merino breed and the males in the cited study.

Cottrell et al. (2008) evaluated the effect of exercise intensity on the lactic acid variable in lambs crossing Border Leicester and Merino, where they found that animals induced to 15 minutes of exercise before slaughter showed an increase of about six times in

circulating levels (13.8 mmol/L) when compared to animals without any effort intensity (3.7 mmol/L).

These studies indicate that this characteristic does not have a fixed pattern of occurrence, and can be influenced by several factors, such as race, sex, pre-slaughter physical activity, and the experiences experienced by the animals during their breeding and growth. In this context, the lactic acid data found in the present study may be a reflection of some stress occurred in the pre-killing of the animals.

Blood glucose, as well as oxygen, is essential for metabolism, being one of the primary energetic substrates for the life process of mammalian cells (Kaneko et al., 2008). Its level is regulated by the release of glycogen deposits, their capture, and peripheral use (Avila, 2013).

The reference values of plasma glucose levels for sheep are between 50mg/dL and 80mg/dL (Kaneko et al., 2008). This variation can happen due to the very efficient homeostatic mechanisms of the body, which involve, for example, endocrine control through insulin and glucagon.

The measured values were within the reference described by Kaneko et al. (2008). The levels obtained by Cottrell et al. (2008) were higher, with an average of 84.68 mg/dL in animals exposed to 15 minutes of exercises before slaughter. The authors reported that plasma glucose can increase about 2 times the normal levels in exercised animals when compared to idle animals.

Teke et al. (2014) observed the effect of density levels on glycemia after transporting animals from a low-density flock (0.27 m<sup>2</sup>/lamb) and obtained lower glucose levels when compared to animals submitted to a high density (0.20 m<sup>2</sup>/lamb). Similarly, Cozar et al. (2016) found that animals under a density of 0.30 m<sup>2</sup>/lamb had the highest glyceimic values before transportation, but after transportation they obtained the lowest glucose values, demonstrating that they used more of their energy sources to adapt. Both works show that the plasmatic glucose activity was influenced by the stress caused by the space subsidy in the transportation.

Lima (2014), evaluating the plasma glucose levels at different pre-slaughter moments, in mestizo animals, observed higher values of glucose in transport (102.1 mg/dL), above the reference limit used in this work. However, in a study conducted by Stempa et al. (2016) lower levels of glucose were verified, in bloodletting, when compared to those of references in animals of the breeds Merino and Dorper.

This can be explained by the fact that animals are subjected to high-stress situations due to pre-slaughter management, since glucose is the primary source of energy for the organism, and the need to mobilize the energy reserves in order to stabilize the feeling of discomfort arises (Lima, 2014).

The hematocrit (Ht) is a blood parameter that indicates the percentage of red cells, in the total volume of blood, being the recommended hematocrit value for the sheep species of 28-40%, for adult and healthy sheep (Reece, 2017a). The hematological values reported for sheep may be influenced by several factors, such as stress, dehydration, and anemia (Pinheiro, Jorge, Miranda-de la lama & Souza, 2015; Madureira, Gomes, Barcelo, Zani, Shecaira, Baccili & Benesi, 2013; Ramos, 2012).

Madureira et al. (2013) found hematocrit values within the normal range for Dorper (37.5%) sheep up to one year old, which were close to the present work. Cozar et al. (2016) reported hematocrit values similar to those found in this work for animals transported at different densities, within the reference values, where they did not differ from each other.

However, Lima, Monteiro, Jorge, Campello, Rodrigues, Viana, Monteiro & Costa (2015) observed values lower than those found in this survey for animals of the Santa Inês breed at 3 to 6 months of age. Pinheiro et al. (2015) in a study with cull ewes evaluated at different times in pre-slaughter (before and after transport and after fasting in the frigorific), found no differences ( $P>0.05$ ) for hematocrit values.

The post-slaughter hematological variables, obtained at the time of bleeding, presented values of glucose and hematocrit within the expected. However, the values measured for lactic acid were above the reference for the species (Table 2).

**Table 2.** Mean, standard deviation and confidence interval (95% CI) of lactic acid (mmol/L), glucose (mg/L), hematocrit (%) of lambs without defined race (SRD) at post-slaughter (n=29).

Variable	Average $\pm$ SD	IC 95%	Reference values*
Lactic acid (mmol/L)	4.81 $\pm$ 1.04	4.43 – 5.19	1.00 – 1.33*
Glucose (mg/dL)	69.79 $\pm$ 10.33	66.03 – 73.55	50 - 80*
Hematocrit (%)	38.93 $\pm$ 3.28	37.74 – 40.13	28 - 40**

\*Kaneko; Harvey; Brass, (2008); \*\*Reece (2017). Source: Authors.

Through the correlation analysis between the studied variables there was a significant correlation only between RR and TR (Table 3), being this low and positive, agreeing with what was observed by Starling et al. (2002), in Corriedale sheep, when they found correlation coefficient values of 0.29 ( $P < 0.01$ ) and with Correa et al. (2012) that noticed a very low correlation, with value of 0.13 ( $P < 0.05$ ).

**Table 3.** Results of Pearson's correlation (r) between pre and post-slaughter parameters in lambs without defined breed (SRD).

	RR	TR	Glucose	Lactic acid	Hematocrit
HR	-0.03	-0.09	0.08	0.08	-0.04
RR	-	0.34*	-0.07	-0.16	0.05
TR	-	-	-0.00	0.25	-0.10
Glucose	-	-	-	0.13	-0.19
Lactic acid	-	-	-	-	-0.14
Hematocrit	-	-	-	-	-

Significance of \* $p < 0.1$ ; HR = Heart rate; RR = Respiratory rate; TR = Rectal temperature. Source: Authors.

Stempa et al. (2016) found a significant, low and positive correlation between lactic acid and glucose in Dorper sheep of 0.37 ( $P < 0.01$ ) and males of 0.38 ( $P < 0.01$ ), however, the correlation was not repeated in Merinos and females, when the values obtained did not present the indicators that reflect the stress are well established in the literature, however, until this moment, they are little explored in the sheep species (Stewart, McGilchrist, Gardner & Pethick, 2018), being necessary to conduct new studies seeking the relationship between the parameters analyzed in this work.

#### 4. Conclusions

Lambs kept under high environmental temperature in the pre-slaughter period present an increase in heart and respiratory rates for the maintenance of homeothermia, a condition proven by the degree of positive and significant association between these parameters.

On the occasion of bleeding in lambs submitted to humane slaughter procedures, there is an intensification of the stress condition reflected by The Increase In The Plasmatic Levels Of Lactic Acid From The Increased Use Of Energetic Substrates.

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