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Influence of lambing on the metabolic profile, neutrophil function and IgG levels in Lacaune sheep. Influência da parição no perfil metabólico, função de neutrófilos e teores de IgG em ovelhas da raça Lacaune.

Michele dos Santos[1*], Marla Schneider[2], Cláudia Medeiros Rodrigues[3], Aline de Jesus da Silva[4], Luana Carolina Bachmann Gregolin[5], Maiara Garcia Blagitz[6], Alice Maria Melville Paiva Della Libera[7], Ana Martiele Engelmann[8], Cássia Bagolin da Silva[9], Davi Fernando Alba[10], Wanderson Adriano Biscola Pereira[11], Marta Lizandra do Rêgo Leal[12]

[1*]- Program Post-Graduate Health, Welfare and Sustainable Animal Production in the Southern Frontier - UFFS, Realeza/PR - Brazil. E-mail: michelefrancheski@gmail.com.
[2]- Central Education Unit Farm College - Chapecó/SC - Brazil. E-mail: marla.schneider.uffs@gmail.com.
[3]- Program Post-Graduate Veterinary Medicine - UFSM, Santa Maria/RG - Brazil.
[4]- Autonomous Veterinarian - São Paulo/SP - Brazil. E-mail: allinejs02@gmail.com.
[5]- Program Post-Graduate Health, Welfare and Sustainable Animal Production in the Southern Frontier - UFFS, Realeza/PR - Brazil. E-mail: lubgregolin@gmail.com.
[6]- Program Health, Welfare and Sustainable Animal Production in the Southern Frontier - UFFS, Realeza/PR - Brazil. E-mail: maiara.azevedo@uffs.edu.br.
[7]- Department of Large Animal Clinic of the FMVZ - USP - São Paulo - Brazil. E-mail: dellalibera@usp.br.
[8]- Program Post-Graduate Veterinary Medicine - UFSM, Santa Maria/RG - Brazil.
[9]- Official Temporary Veterinarian, São Borja/RS - Brazil. E-mail: cassiabagolin@hotmail.com.
[10]- Autonomous Veterinarian - Chapecó/SC - Brazil. E-mail: davi.alba@hotmail.com.
[11]- Federal Institute of Santa Catarina, Concórdia, Santa Catarina, Brazil. E-mail: wanderson.pereira@ifc.edu.br.
[12]- Department of Large Animal Clinic of the Federal University of Santa Maria - UFSM, Santa Maria/RG - Brazil. E-mail: martalizandra@gmail.com.

Abstract

The objective of this study was to evaluate the metabolic profile, the activity of blood neutrophils, and the immunoglobulin G levels of primiparous and multiparous sheep (Lacaune breed) during the first 30 days after lambing. Fifteen primiparous ewes (GPR) and 15 multiparous ewes (GPM) were used. Evaluations were performed on the days of lambing and at three, seven, fifteen and thirty days post-lambing. In general, the basal and bactericidal activity of neutrophils were lower in GPR than in GPM. Phagocytosis was greater in the primiparous sheep in the initial moments after lambing. Non-esterified fatty acid concentrations were highest for GPM, indicating a negative energy balance in this group. The results obtained in this study allow us to conclude that primiparous sheep have higher neutrophil phagocytosis, while this cell type has higher bactericidal activity in multiparous sheep. Multiparous ewes presented with higher lipomobilization due to maintenance needs and higher milk production.
Keywords: Energetic Metabolism. Neutrophils. Primiparous. Multiparous. Postpartum.

Resumo

O objetivo do presente estudo foi avaliar o perfil metabólico, a atividade de neutrófilos sanguíneos e os teores de imunoglobulina G de ovelhas primíparas e multíparas da raça Lacaune durante os primeiros 30 dias após o parto. Foram utilizadas 15 ovelhas primíparas (GPR) e 15 ovelhas multíparas (GPM). Foram realizadas avaliações nos dias do parto e aos três, sete, quinze e trinta dias pós-parto. No geral a atividade basal e bactericida dos neutrófilos foi menor no GPR do que no GPM. E nos momentos iniciais ao parto, a fagocitose foi maior para as ovelhas primíparas. Os AGNES apresentaram as maiores concentrações para as GPM indicando um balanço energético negativo nesse grupo. Os resultados obtidos nesse estudo nos permite concluir que ovelhas primíparas possuem maior fagocitose de neutrófilos enquanto que as multíparas possuem maior atividade bactericida desse tipo celular. Ovelhas multíparas apresentam maior lipomobilização em decorrência das necessidades de mantença e maior produção de leite.
Palavras-chave: Metabolismo energético. Neutrófilos. Primíparas. Multíparas. Pós-parto.

Introduction

Lacaune ewes are major milk producers (BRITO et al., 2006) and, as in dairy cows the period around lambing and post-lambing is challenging for these females (ARAÚJO et al., 2014; PICCIONE et al., 2009). Expressive physiological, nutritional, metabolic, and endocrine changes occur and can generate disease in these animals, which negatively affects their performance, health, and welfare (CABIDDU et al., 2020, HERNÁNDEZ-CASTELLANO et al., 2019). It is estimated that the incidence of disease and the annual rate of losses (deaths) are around 5% to 10%, with such losses occurring more frequently (MAVROGIANNI; BROZOS, 2008) in the peripartum period, i.e., at or after delivery (MAVROGIANNI; BROZOS, 2008).

One of the most important factors in triggering metabolic diseases is the negative energy balance (BEN), resulting from the high energy demand at the end of pregnancy and insufficient matter intake (GONZÁLEZ; SILVA, 2006; PEIXOTO; OSÓRIO, 2007). Depending on the intensity, BEN can cause serious metabolic diseases (CABIDDU et al., 2020).

Moreover, during the peripartum period, ruminants are characterized by an immunological deregularization condition (TREVISI; MINUTI, 2018; HERNÁNDEZ-CASTELLANO et al., 2019; SUCUPIRA et al., 2019; SANTOS et al., 2020). Regarding innate immunity, neutrophils are a major line of defense in the body (SOUZA et al., 2012) and studies have shown that primiparous sheep and cows have a higher intensity of phagocytosis compared to multiparous females (SUCUPIRA et al., 2019; MEHRZAD et al., 2009). In the peripartum period, bovine and ovine females also have lower humoral immune responses (TREVISI; MINUTI, 2018; et al., HERNÁNDEZ-CASTELLANO et al., 2019). However, few studies have evaluated the levels of antibodies in sheep in the peripartum period.

The metabolic profile, leukocyte phagocytosis ability, and the quantification of immunoglobulins can be useful tools to investigate the possible adaptation of dairy sheep to the conditions typical of extensive or intensive production in the peripartum period. Previous studies generally focused on the lactation period (CASTILLO et al., 2016), while research on metabolic adaptations around the peripartum period was investigated less frequently, particularly in sheep with different parities. This critical physiological phase has been widely studied and documented in dairy cows (LOOR et al., 2013; LOPREIATO et al., 2019), but few studies on sheep and goat species exist.

In view of this, the objective of the present study was to evaluate the metabolic profile, the oxidative metabolism and phagocytosis of neutrophils, and the levels of light and heavy chain immunoglobulin G in primiparous and multiparous dairy sheep for 30 days after lambing.

Materials and methods

Research Ethics Committee

The study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Southern Border (UFFS) (No. 4143140619).

Animals used, nutritional management and facilities

The experiment was conducted at a dairy sheep farm located in Chapecó, Santa Catarina, Brazil. Blood samples were collected from 30 sheep of the Lacaune breed, which were divided into two groups. The first group consisted of 15 primiparous ewes, and the second group was of 15 multiparous ewes. During the study, each animal received 0.8 kg/day of concentrate, distributed in

two doses/day (at 7:00 and 18:00). The animals also received 3.6 kg of corn silage per day and 0.25 kg/animal/day of tifton hay, totaling a diet with 22% crude protein. They were also supplied with water *ad libitum*. During the entire study period, the females were housed in a covered shed, confined to side-by-side collective stalls of 12 m^2 and bedding of razors, with an average of 20 animals allocated per stall. Two milkings were performed per day (at 05:00 and 17:00) using a side-by-side milking system. The average milk production of the primiparous ewes was 1.5 L/animal/day, while the average milk production of the multiparous ewes was 2.0 L/animal/day. After birth, the lambs stayed with their mothers for 24 to 48 hours and then went to the nursery with artificial feeding. Before the beginning of the experimental period, all sheep were submitted to physical examination to rule out possible diseases (FEITOSA et al., 2014) so that diseased animals could be excluded from the study.

Blood collection and experimental moments

Blood samples were collected from the vein in Vacutainer®-type vials with heparin anticoagulant for the evaluation of the oxidative metabolism of neutrophils, while blood samples were collected in Vacutainer®-type vials without anticoagulants for measuring the metabolic parameters. All collections were performed before the morning feeding.

The collections were performed at the following times: on the day of delivery (Moment 1), three days after delivery (Moment 2), seven days after delivery (Moment 3), 15 days after delivery (Moment 4), and 30 days after delivery (Moment 5). The beta-hydroxybutyrate (BHBA) samples were collected at the following times: three days after delivery (Moment 1), 15 days after delivery (Moment 2), and 30 days after delivery (Moment 3).

Biochemical analysis

In the laboratory, the samples were centrifuged at 3,000 rpm to obtain the serum. Then, the serum samples were dosed with triglycerides (Bioclin- Ref. K083-2), low density lipoprotein (LDL) (Bioclin- Ref. K088-27), high density lipoprotein (HDL) (Bioclin- Ref. K071-23), calcium (Bioclin- Ref. K051-2), fructosamine (Bioclin- Ref. K135-4), albumin (Bioclin- Ref. K040-1), and non-esterified fatty acids (AGNES) (Randox- Ref. FA 115). All analyses were performed in a Mindray automatic biochemical analyzer - BS 120.

Beta-hydroxybutyrate

Beta-hydroxybutyrate was evaluated using a portable digital meter (FreeStyle Optium Neo®) and analyzed from the animal's whole blood. To perform the test, after the blood was collected from the jugular vein of sheep into tubes without anticoagulant, 1.5 μl of this blood was pipetted in the laboratory and deposited on tape for the immediate reading of the BHBA levels, which were expressed in mmol/L.

Electrophoresis

For the fractionation of proteins, electrophoresis in polyacrylamide gel containing sodium dodecyl sulfate was used, as described by Fagliari et al. (2006), in mini gel (10 x10 cm). After the end of the running time, the gel was stained with Coomassie Blue until the bands were marked, and the excess of the dye was removed by the 7% acetic acid solution. The gels were later photographed, and the identification and quantification of the protein fractions was performed with Labimage1D (Loccus Biotechnology) software. A standard containing fractions with molecular weights between

10 and 250 KD (Kaleidoscope - BIORAD) was used as the reference for the identification of the protein fractions. For protein quantification, the total protein content previously obtained by the Biuret technique was used as a reference. The fractions quantified were heavy chain immunoglobulin G (IgG) and light chain IgG.

Functional evaluation of neutrophils

The evaluation of the oxidative metabolism of neutrophils was performed by the nitroazole tetrazolium (NBT) technique with a commercial kit (cat. N. 0329, AMRESCO, Solon, USA) using the cytochemical method (Park; Good, 1970). Here, 50 µl of 1.5% NBT solution was homogenized to 1 ml of heparinized blood in a test tube in the unstimulated technique (NBT-NE), and 50 µl of 1.5% NBT solution was homogenized to 1 ml of heparinized blood and 10 µl of stimulant (zymosan A de *Saccharomyces cerevisiae,* cat. N. Z4250, Sigma Aldrich, St. Louis, USA) in another test tube for the stimulated technique (NBT-E). Both tubes were incubated at 37°C for 10 minutes. Blood smears were then prepared and stained with rapid Panoptic dye. One hundred neutrophils were counted on each of the stained blood smears using an optical microscope with an immersion objective (100X). For NBT-E and NBT-NE, neutrophils that reduced nitroazole tetrazolium were considered positive, i.e., neutrophils that presented cytoplasmic granules with a violet or blackish color (formazan crystals), independent of the number and size of the granulations. In the stimulated technique, the neutrophils that showed particles of zymosan inside were considered positive for phagocytosis.

Statistical analysis

The data were evaluated for normality and homoscedasticity by the Shapiro-Wilk test. Then, a repeated measures Analysis of Variance (ANOVA) test was applied, followed by the Student-Newman-Keuls test for comparison between groups and moments. The value of $P \leq 0.05$ was considered significant.

Results and discussion

Data for albumin, total protein, light chain IgG and heavy chain IgG levels are presented in Table 1.

Albumin is the most abundant protein in plasma and its serum values demonstrate the animal's long-term protein status (PEIXOTO; OSÓRIO, 2007) due to the low speed of the synthesis and degradation of this molecule (MARTIN et al., 2004). In our study, performed over 30 days after delivery, there was no difference in the albumin levels among groups and experimental moments ($P>0.05$). Slavov et al. (2018) also did not observe any difference in the albumin concentrations between primiparous and multiparous Lacaune sheep. According to Contreras et al. (2000), albumin tends to decrease after calving and peak lactation due to milk production. It is possible that due to the percentage of protein offered in the diet of these females, albumin changes did not occur in this study. Total proteins are the best indicators of the body's protein reserves (MIGLIO et al., 2015). In this study, there was no difference in the total protein levels ($P>0.05$). However, Slavov et al. (2018) identified a slight increase in the total proteins for multiparous sheep compared to primiparous sheep. Other studies detected a decrease in the total protein values in the immediate postpartum period (SOARES et al., 2014; SILVA et al., 2013), with a recovery of the mean values around 28 days

postpartum (SOARES et al., 2014). The values of this biochemical variable measured in the current study were higher than those reported in the literature (6.0 mg/L–7.9 mg/L) (KANEKO et al., 2008).

Table 1 - Serum (g/dL) concentration of total protein, albumin, immunoglobulin G (IgG; heavy and light chain), and calcium (Ca) evaluated in Lacaune sheep in the postpartum period.

Group	Moments	Protein	heavy IgG	light IgG	Albumin	CA
1	1	9,07 ±0,31[Aa]	0,92 ± 0,07[Aa]	1,49 ± 0,17[Aa]	2,33 ± 0,19[Aa]	7,97 ± 0,49[Aa]
1	2	10,53 ±0,57[Aa]	1,00 ± 0,08[Aa]	1,36 ± 0,13[Aa]	2,23 ± 0,20[Aa]	7,50 ± 0,49[Aa]
1	3	9,92 ±0,29[Aa]	1,11 ± 0,08[Aa]	1,42 ± 0,17[Aa]	2,38 ± 0,19[Aa]	7,90 ± 0,49[Aa]
1	4	9,70 ±0,47[Aa]	1,00 ± 0,07[Aa]	1,25 ± 0,15[Aa]	2,13 ± 0,20[Aa]	7,27 ± 0,53[Aa]
1	5	10,24 ±0,25[Aa]	1,03 ± 0,09[Aa]	1,55 ± 0,09[Aa]	2,08 ± 0,22[Aa]	7,21 ± 0,53[Aa]
2	1	8,88 ± 0,41[Aa]	0,88 ± 0,07[Aa]	0,86 ± 0,08[Aa]	2,45 ± 0,11[Aa]	7,92 ± 0,24[Aa]
2	2	9,56 ± 0,42[Aa]	0,98 ± 0,10[Aa]	0,83 ± 0,06[Aa]	2,36 ± 0,14[Aa]	8,10 ± 0,19[Aa]
2	3	9,64 ± 0,84[Aa]	0,99 ± 0,05[Aa]	1,02 ± 0,12[Aa]	2,36 ± 0,13[Aa]	7,88 ± 0,22[Aa]
2	4	9,71 ± 0,49[Aa]	0,97 ± 0,05[Aa]	1,38 ± 0,19[Aa]	2,52 ± 0,08[Aa]	8,37 ± 0,20[Aa]
2	5	9,28 ± 0,51[Aa]	0,91 ± 0,10[Aa]	1,16 ± 0,23[Aa]	2,56 ± 0,12[Aa]	8,12 ± 0,29[Aa]

Results expressed as mean ± standard error of the mean. Capital letters indicate a statistically significant difference ($P<0.05$) between groups; lower case letters indicate statistical difference ($P\geq0.05$) between moments within the same group. Group 1: Primiparous ewes; Group 2: Multiparous ewes. Moment 1: day of lambing; Moment 2: three days after lambing; Moment 3: seven days after lambing; Moment 4: 15 days after lambing; Moment 5: 30 days after lambing.

During the 30 days of the study, the Lacaune sheep did not clinically present any disease, so this increase in the mean protein values in both groups could be related to the total protein supply in the diet, which was 22% per day throughout the trial period. This value of crude protein offered to the sheep in the peripartum period was higher than that offered in other studies (PICCIONE et al., 2009; SCHMITT et al., 2018), and according to Rankins (2005), sheep in the lactation period need a diet with about 13.5% crude protein.

It is important to note that one of the difficulties encountered in correlating the data is due to the limited literature available with different reference ranges, many of which are not always applicable, since one must take into account the aptitude, food management, and environment, among other characteristics of these animals (SOARES et al., 2014).

The light and heavy chain IgG values were also not influenced by parturition or experimental moments ($P>0.05$). Hernández-Castellano et al. (2019) detected a lower concentration of serum IgG around lambing in multiparous sheep, which gradually increased and reached its maximum at 40 days of lactation. Studies correlating the parity factor with IgG concentrations were performed with Dutch sheep and cows (TABATABAEI et al., 2013; HERR et al., 2011). In these studies, the authors also observed no difference in the IgG levels.

The data obtained suggest that there is no influence of parturition on IgG values, and that both primiparous and multiparous ewes may guarantee an effective humoral immune response to some infection. Furthermore, this IgG production is vital because the same blood plasma derivative is incorporated into the epithelial cells of the mammary gland (BUOGOYONE; DUNCAN, 1998) to form the colostrum.

Another important factor with a close link to immunity is calcium. This mineral acts as the secondary messenger of different cells of the immune system (IZQUIERDO et al., 2014), and its intracellular concentration promotes the activation of oxidative metabolism for neutrophils (PENFIELD; DALE, 1984). Calcium is also of extreme importance for the production of colostrum

and milk. Therefore, low calcium levels in the postpartum period may predispose females to infectious diseases, in addition to hypocalcemia (GONZÁLEZ; SILVA, 2006).

In our study, the calcium values remained constant in the 30 days postpartum and did not differ between groups and within each group ($P > 0.05$). Silva et al. (2013) also did not detect any difference in the calcium concentrations in multiparous Santa Inês sheep during the postpartum period. However, when compared with the reference range, both groups in the current study were below the values referenced in the literature (KANEKO et al., 2008), indicating that both primiparous and multitudinous sheep had a high calcium demand during parturition and lactation.

The results of oxidative metabolism (reduction of nitroazole tetrazolium) and phagocytosis of zymosan particles by blood neutrophils of primiparous and multiparous Lacaune sheep are presented in Table 2.

Regarding the basal oxidative metabolism (MOB), the primiparous sheep had a lower percentage of neutrophils on the day of lambing (M1) and at three days after lambing (M2) than the multiparous sheep at seven (M3) and 15 days after lambing (M4), while the multiparous sheep had a higher percentage of neutrophils at 30 days after lambing (M5) than the primiparous sheep ($P \leq 0.05$).

In the primiparous group, there was a lower percentage of neutrophils at the time of delivery (M1) and three days after delivery (M2 than 15 days after delivery (M4). In the multiparous group, there was a lower percentage of neutrophils at M1 and M2 than all other times. At seven days (M3), the percentage was higher than 15 days (M4) and 30 days after delivery (M5), while at 15 days (M4) the percentage was also higher than at 30 days after delivery (M5) ($P \leq 0.05$). In the literature, only one study did not report a difference in the MOB between primiparous and multiparous sheep (SANTOS et al., 2020).

Both the primiparous and the multiparous ewes exhibited lower MOB in the early moments of the postpartum period. A lower percentage of MOB soon after delivery was also detected by Fonteque et al. (2013). The assessment of the MOB indicates the presence of blood neutrophils. Thus, lower percentages of this polymorphonuclear indicator in these periods may be related to the flow of neutrophils to the uterus (FERNANDES et al., 2013) and mammary glands.

As for the MOE, the multiparous ewes had a higher percentage of neutrophils reactive to NBT at seven days after delivery than the primiparous ewes at all experimental times ($P \leq 0.05$) (Table 2). Regarding the moments within the groups, the primiparous ewes at M1 and M2 had a lower percentage of neutrophils reactive to NBT compared with ewes at M3, M4, and M5. The multiparous ewes showed a higher percentage of neutrophils reactive to NBT at seven days after delivery (M3) compared to other experimental days ($P \leq 0.05$) (Table 2).

The MOE results diverged from those reported in the literature. Fonteque et al. (2013) reported higher activity of neutrophils reactive to NBT in multiparous ewes, a result also observed in our study. However, Santos et al. (2020) did not observe a difference in the EOM among sheep regardless of the number of lambings. By using the NBT reduction test we can indirectly measure the intracellular production of ERO by the neutrophils. The production of these is essential to ensure the microbiocidal activity of the neutrophils; therefore, lower percentages of neutrophils reactive to NBT (SANTOS et al., 2020) soon after lambing in both multiparous and primiparous animals may indicate that these animals had a deregularization of their immune responses, which may predispose sheep to infectious diseases at the beginning of lactation.

Two groups were considered for neutrophil phagocytosis: stimulated/zymosan and zymosan. In both the stimulated/zymosan and zymosan groups, the multiparous groups presented, at seven days

(M3), a lower percentage of phagocytosis in relation to the primiparous groups at all experimental times ($P \leq 0.05$) (Table 2).

Between the moments and in the presence of zymosan, the primiparous sheep had less phagocytic activity at three days (M2) than at 30 days (M5), while the multiparous sheep had less phagocytic activity at seven days (M3) than at 30 days (M5) postpartum ($P \leq 0.05$).

For the stimulated/zymosan treatment group, there was only a significant difference in the multiparous sheep. In the evaluation of neutrophil phagocytosis and with zymozan, within each group, the multiparous ewes presented with a lower power of phagocytosis at M3 compared with the other moments ($P \leq 0.05$). There was no significant difference within the primiparous group.

The reduced phagocytic activity by neutrophils soon after lambing is well established in sheep and cattle (SANTOS et al., 2020; SUCUPIRA et al., 2019; MEHRZAD et al., 2009). Results similar to our study also indicated that primiparous females had higher postpartum phagocytosis intensity in relation to multiparous females (SUCUPIRA et al., 2019; MEHRZAD et al., 2009). The results of the NBT test indicated that lambing negatively affects phagocytosis, as was already mentioned by other authors (KIMURA et al., 2006; SUCUPIRA et al., 2019; SANTOS et al., 2020). This deregularization early in lactation may occur, in part, due to deficiencies in the energy, protein, and minerals necessary for the composition and maintenance of the immune system (GOFF; KIMURA, 2009). In this study, calcium in both groups of ewes was below the reference values. This mineral has a close connection with the activation and function of immune cells (KIMURA et al., 2006), including phagocytosis, and may therefore be one of the causes for the lower phagocytosis intensity during the lactation of these sheep.

Table 2 - Percentage of oxidative metabolism (reduction of nitroazole tetrazolium) and phagocytosis of zymosan particles by the blood neutrophils of Lacaune sheep in the postpartum period.

Group	Moment	Basal	Stimulated	Stimulated/zymosan	*Zymosan*
1	1	$8{,}20 \pm 0{,}79^{Bb}$	$10{,}73 \pm 1{,}43^{Bb}$	$35{,}60 \pm 2{,}54^{Aa}$	$27{,}60 \pm 2{,}34^{Aab}$
1	2	$9{,}40 \pm 0{,}92^{Bb}$	$14{,}80 \pm 1{,}06^{Bb}$	$35{,}00 \pm 2{,}55^{Aa}$	$25{,}53 \pm 1{,}94^{Ab}$
1	3	$12{,}93 \pm 1{,}54^{Aab}$	$17{,}66 \pm 1{,}09^{Ba}$	$30{,}40 \pm 1{,}76^{Aa}$	$34{,}40 \pm 3{,}34^{Aab}$
1	4	$15{,}00 \pm 0{,}95^{Aa}$	$21{,}06 \pm 2{,}01^{Ba}$	$29{,}53 \pm 2{,}24^{Aa}$	$31{,}53 \pm 1{,}91^{Aab}$
1	5	$13{,}20 \pm 1{,}10^{Aab}$	$18{,}14 \pm 0{,}72^{Ba}$	$31{,}93 \pm 2{,}09^{Aa}$	$35{,}26 \pm 1{,}88^{Aa}$
2	1	$11{,}33 \pm 0{,}79^{Bd}$	$16{,}46 \pm 0{,}82^{Bb}$	$33{,}93 \pm 0{,}89^{Aa}$	$26{,}93 \pm 1{,}64^{Aab}$
2	2	$11{,}33 \pm 0{,}65^{Bd}$	$16{,}60 \pm 0{,}75^{Bb}$	$31{,}60 \pm 0{,}68^{Aa}$	$26{,}46 \pm 1{,}06^{Aab}$
2	3	$28{,}80 \pm 1{,}95^{Aa}$	$28{,}73 \pm 2{,}45^{Aa}$	$20{,}06 \pm 2{,}23^{Bb}$	$20{,}40 \pm 1{,}76^{Bb}$
2	4	$21{,}20 \pm 1{,}31^{Ab}$	$20{,}13 \pm 1{,}40^{Bb}$	$30{,}26 \pm 2{,}03^{Aa}$	$28{,}40 \pm 2{,}26^{Aab}$
2	5	$16{,}93 \pm 1{,}35^{Ac}$	$17{,}06 \pm 1{,}39^{Bb}$	$31{,}66 \pm 1{,}64^{Aa}$	$29{,}20 \pm 2{,}2^{Aa}$

Results expressed as mean ± standard error of the mean. Capital letters indicate a statistically significant difference ($P \leq 0.05$) between groups; lower case letters indicate a statistically significant difference ($P \leq 0.05$) between moments within the same group. Group 1: primiparous ewes; Group 2: multiparous ewes. Moment 1: day of lambing; Moment 2: three days after lambing; Moment 3: seven days after lambing; Moment 4: 15 days after lambing; Moment 5: 30 days after lambing.

The results of the variables of the metabolic profile of Lacaune sheep are presented in Tables 3 and 4. Statistical differences in the cholesterol, HDL and LDL values ($P \leq 0.05$) were observed. However, there was no significant difference in the serum concentrations of fructosamine and triglycerides among groups and experimental moments ($P > 0.05$).

There was no significant difference in cholesterol levels between the groups ($P \leq 0.05$). In the primiparous sheep, higher cholesterol levels were detected at M3 compared with M4 ($P \leq 0.05$). The

serum cholesterol concentrations of the multiparous sheep remained below the reference range (52–76 mg/dL) (KANEKO et al., 2008), but there was no difference between the experimental moments (P>0.05).

In a study by Pesántez-Pacheco et al. (2019), it was observed that both multiparous and primiparous sheep had cholesterol values within the expected range soon after lambing. However, there was a reduction in the cholesterol levels in multiparous ewes at postpartum. In another study where only multiparous sheep were analyzed, the authors also observed a reduction in the cholesterol levels in the postpartum period (PICCIONE et al., 2009). This decrease over the time near the peak of lactation can be attributed to increased cholesterol uptake for milk synthesis, which is more pronounced in multiparous sheep, since milk production is higher in these ewes when compared with primiparous ewes (NAZIFI et al., 2002).

No difference in HDL levels between the groups was observed (P>0.05). Within the groups, the primiparous sheep had lower concentrations of HDL at the time of lambing (M1) and three days after lambing (M2) compared with seven, 15 and 30 days (M3, M4, and M5) postpartum ($P \leq 0.05$). The multiparous ewes presented with lower concentrations of HDL in M1 compared with all other moments. At 30 days postpartum, this group showed higher mean values compared with other times of the study ($P \leq 0.05$). Both primiparous and multiparous ewes showed higher values of HDL at the end of the experiment.

There was also no significant difference between the groups regarding the LDL levels (P>0.05). The primiparous sheep had higher and lower LDL values, respectively, at seven and 15 days after delivery ($P \leq 0.05$). The multiparous ewes showed higher concentrations of LDL on the day of lambing compared with the other experimental times ($P \leq 0.05$).

In sheep's blood, most of the cholesterol is linked to HDL, which represents 76% of the circulating lipoproteins (LEAT et al., 1976). It is important to note that there are few studies on these lipoproteins in the postpartum period in dairy sheep (NAZIFI et al., 2002; MUSA, 2020) and no other study differentiating the behavior of these lipoproteins between primiparous and multiparous sheep.

The data found in the literature indicate that multiparous dairy sheep have a lower concentration of HDL and LDL from lambing up to 2–3 weeks postpartum, gradually increasing from lactation (NAZIFI et al., 2002). In the present study, HDL concentrations were the lowest concentrations soon after lambing for both groups, while the lowest LDL concentrations were observed at the beginning of the peak of lactation (3–4 weeks postpartum) in both experimental groups.

In a study carried out with primiparous cows, both the HDL and LDL decreased on the day of calving, with values gradually increasing until the peak of lactation (KURPINSKA et al., 2015). This increase in HDL during lactation can be explained by the effect of VLDL catabolism and cholesterol ester synthesis, which increase HDL production (MAZUR; RAYSSIQUIER, 1988), while the increase in LDL soon after calving may be related to a decrease in estrogen levels, causing a decrease in LDL receptors and thus an increase in the plasma concentration of LDL (BAUCHART, 1993).

Although there were no statistically significant differences in the triglyceride contents between and within groups (P>0.05), the mean values from ewes in both groups were below the reference values (17.6–24.0 mg/dL) (KANEKO et al., 2008). Some studies found postpartum triglyceride values decreased for both primiparous and multiparous ewes (GONZÁLEZ-GARCÍA et al., 2015; PESÁNTEZ-PACHECO et al., 2019). However, Brito et al. (2006) found no reduction in triglyceride levels in multiparous ewes during lactation.

Triglycerides, which accumulate in the fat tissue, are an important source of metabolic energy (GONZÁLEZ, SILVA 2006) and are consumed when energy needs increase, such as postpartum

(PESÁNTEZ-PACHECO et al., 2019). They are also used for the synthesis of milk fat (MUNDIM et al., 2007). Our study indicates that both groups mobilized triglycerides, either as a source of energy or for the composition of milk fat, since the sheep presented values below the reference range.

There were also no significant differences in triglyceride concentrations between the groups and within each group ($P > 0.05$). Silva et al. (2013), when analyzing multiparous Santa Inês sheep, found a slight decline in the mean values during the period up to 90 days after lambing. Meanwhile, Soares et al. (2014) did not observe such declines in their study of Dorper multiparous ewes.

Fructosamine is a stable ketoamine that is formed by a glucose reaction with amine groups of proteins, mainly albumin (SILVA et al., 2013). As the half-life of albumin is approximately 20 days, the measurement of this variable becomes important for the evaluation of glucose in the last two to three weeks without variations in its values (KANEKO et al., 2008), making it the most reliable parameter for the evaluation of glucose metabolism. Thus, in this study, we observed (through measuring fructosamine) that there was no significant difference in the glucose of the groups over the course of 30 days.

Table 3 - Serum concentrations (mg/dL) of total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and fructosamine (µmol/L) evaluated in Lacaune sheep in the post calving period.

Group	Moment	Cholesterol	Triglycerides	HDL	LDL	Fructosamine
1	1	58,86± 6,03[Aab]	19,40± 2,19[Aa]	39,95± 1,85[Ac]	21,93± 2,30[Ab]	176,30± 14,57[Aa]
1	2	60,13± 6,06[Aab]	18,50± 1,86[Aa]	40,64± 1,36[Ac]	26,37± 2,47[Aa]	180,33± 20,39[Aa]
1	3	66,80± 5,86[Aa]	19,13± 1,82[Aa]	46,06± 1,21[Ab]	22,15± 1,83[Ab]	180,44± 13,94[Aa]
1	4	53,86± 5,53[Ab]	14,57± 1,72[Aa]	44,71± 3,55[Aab]	13,95± 1,79[Ac]	159,06± 15,35[Aa]
1	5	57,53± 7,25[Aab]	16,42± 1,76[Aa]	49,10± 4,06[Aa]	18,18± 2,81[Ab]	163,90± 17,34[Aa]
2	1	45,40± 3,51[Aa]	15,64± 1,46[Aa]	29,09± 2,65[Ac]	16,06± 1,98[Aab]	149,66± 5,87[Aa]
2	2	49,46± 4,55[Aa]	17,00± 1,04[Aa]	34,40± 2,53[Ab]	19,79± 2,26[Aa]	147,35± 8,19[Aa]
2	3	44,60± 4,66[Aa]	14,50 ± 1,62[Aa]	35,18± 3,57[Ab]	12,02± 1,64[Ab]	146,41± 6,65[Aa]
2	4	45,20± 3,84[Aa]	15,28± 1,02[Aa]	36,15± 3,84[Ab]	10,57± 1,78[Ab]	154,56± 8,23[Aa]
2	5	54,80± 5,30[Aa]	19,78± 2,07[Aa]	47,31± 4,94[Aa]	11,08± 1,44[Ab]	162,93± 9,89[Aa]

Results expressed as the mean ± standard error of the mean. Capital letters indicate a statistically significant difference ($P > 0.05$) between groups; lower case letters indicate a statistically significant difference ($P \leq 0.05$) between moments within the same group. Group 1: primiparous ewes; Group 2: multiparous ewes. Moment 1: day of lambing; Moment 2: three days after lambing; Moment 3: seven days after lambing; Moment 4: 15 days after lambing; Moment 5: 30 days after lambing.

The results of BHBA and AGNES are expressed in Table 4. There was no difference in the BHBA values between groups and within each experimental group ($P \leq 0.05$). The BHBA levels remained within the reference values for the species (0.36–0.80 mmol/L) (HARMEYER; SCHLUMBOHM, 2006). González-García et al. (2015) observed that primiparous ewes showed higher concentrations of BHBA at all evaluated times compared with multiparous ewes. However, when analyzing primiparous and multiparous ewes, Pesántez-Pacheco et al. (2019) detected higher BHBA values in multiparous ewes. Beta-hydroxybutyrate is one of the main ketone bodies, and the increase in its serum concentration occurs when the amount of AGNES exceeds the oxidation capacity of the liver (DRACKLEY, 1999). The results indicate good adaptation by both groups evaluated, since the intensity of the negative energy balance is related to the intensity of AGNE mobilization and, consequently, to the production of BHBA, which remained within the reference range at all times during the study (DRACKLEY, 1999).

As for AGNES, higher levels were observed in M1 and M2 in multiparous ewes compared with the same experimental moments of the primiparous ewes (Table 4) ($P \leq 0.05$). Within the groups,

higher values of AGNES were detected one the day of lambing (M1) and at three days postpartum (M2) in multiparous sheep compared with M3, M4, and M5. However, there was no significant difference in the concentrations of AGNES within the primiparous group. The highest mean value was obtained for the multiparous ewes on the day of delivery (0.90 mmol/L), which was above the reference values found in the literature (0.18–0.68 mmol/L) (HARMEYER; SCHLUMBOHM, 2006).

The AGNES levels reflect the level of lipid catabolism in the fat stores and its concentration increases when glucose metabolism is deficient or when there is a high demand for energy (DRACKLEY, 1999). In the present study, the levels of AGNES in the multiparous ewes up to three days after lambing reflected this lipidic catabolism, signaling that these animals went through a moderate negative energy balance, since the values of BHBA were within the reference range (PEIXOTO; OSÓRIO, 2007). Pesántez-Pacheco et al. (2019) found AGNES values within the reference range for both primiparous and multiparous sheep at the end of gestation until postpartum and, as in our study, it was the multiparous sheep that underwent a greater challenge of energetic adaptation. It is worth noting that the multiparous sheep produced an average of 2.0 kg of milk per day and the primiparous sheep produced 1.5 kg/day, which resulted in a higher energy demand for maintenance and milk production in the multiparous sheep. As a consequence, AGNES must have been used by the various tissues as an alternative source of energy.

Unlike the results cited above, González- García et al. (2015) identified that the values of AGNES in primiparous ewes were substantially higher (0.9 mmol/L) than in the group of multiparous ewes (0.5 mmol/L) at postpartum. This difference may be linked to the age of the first mating of the primiparous ewes in the above mentioned study, as they may have been finishing their own growth while meeting the needs of the fetus at the same time, resulting in higher nutrient requirements (GARDENER et al., 2007).

Furthermore, the data for AGNES showed that the peak lactation starting at 30 days after lambing (BRITO et al., 2006) did not influence the results, which may suggest that the metabolic challenges were greater at lambing, especially for sheep with more reproductive cycles.

Table 4 - Serum concentrations (mmol/L) of beta-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) evaluated in Lacaune sheep in the postpartum period.

Group	Moment	NEFA	BHBA
1	1	$0,52\pm 0,07^{Ba}$	$0,46\pm 0,04^{Aa}$
1	2	$0,51\pm 0,08^{Ba}$	$0,37\pm 0,04^{Aa}$
1	3	$0,37\pm 0,05^{Ba}$	$0,31\pm 0,03^{Aa}$
1	4	$0,26\pm 0,04^{Ba}$	
1	5	$0,23\pm 0,02^{Ba}$	-
2	1	$0,90\pm 0,10^{Aa}$	$0,57\pm 0,06^{Aa}$
2	2	$0,82\pm 0,09^{Aa}$	$0,40\pm 0,03^{Aa}$
2	3	$0,37\pm 0,06^{Bb}$	$0,38\pm 0,04^{Aa}$
2	4	$0,39\pm 0,05^{Bb}$	-
2	5	$0,20\pm 0,01^{Bb}$	-

Results are expressed as the mean $\pm$ standard error of the mean. Capital letters indicate a statistically significant difference ($P\leq0.05$) between groups; lower case letters indicate a statistically significant difference ($P\leq0.05$) between moments within the same group. Group 1: primiparous ewes; Group 2: multiparous ewes. AGNES Moments - Moment 1: day of lambing; Moment 2: three days after lambing; Moment 3: seven days after lambing; Moment 4: 15 days after lambing; Moment 5: 30 days after lambing. BHBA Moments - Moment 1: three days after the birth; Moment 2: 15 days after the birth; Moment 3: 30 days after the birth.

Conclusion

The results obtained in this study allowed us to conclude that primiparous sheep have higher neutrophil phagocytosis, while multiparous sheep neutrophils have higher bactericidal activity. Multiparous ewes presented with higher lipomobilization due to higher maintenance needs and milk production.

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