Research, Society and Development, v. 9, n. 11, e46791110020, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.10020

Inclusion of pepper extract containing capsaicin in the diet of ewes in the mid-lactation period: effects on health, milk production, and quality

Inclusão de extrato de pimenta contendo capsaicina na dieta de ovelhas no período médio de lactação: efeitos na saúde, produção e qualidade do leite

Inclusión de extracto de pimiento que contiene capsaicina en la dieta de las ovejas en el período de lactancia media: efectos sobre la salud, la producción de leche y la calidad

Received: 11/08/2020 | Reviewed: 11/11/2020 | Accept: 11/18/2020 | Published: 11/24/2020

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Abstract

Pepper extract (PE, 5 g capsaicin/kg PE) was added to the feed of the sheep during the lactation period (Day 75-93) to maintain production, improve milk quality, and preserve their health. The groups were: T0, control, (without PE); T200 (200 mg PE/kg concentrate) and T400 (400 mg PE/kg concentrate). The reduction in milk production (L) was smaller in the T400 ewes on days 0 to 18 and 14 to 18 than in the T0 group. Feed conversion was lower in sheep in groups T200 and T400 than in group T0. The interaction between the treatment and the day for protein, lactose and total milk totals was greater in ewes that consumed PE on day 18. The somatic cell counts in milk were lower in the T400 ewes. The levels of total protein and globulin were the highest in the blood of animals in the T400 group. There were lower levels of reactive oxygen species and lipoperoxidation in the serum and milk of animals in groups T200 and T400. On the 18th day, the serum of sheep that consumed PE increased

levels of non-protein thiols and superoxide dismutase activities. The inclusion of PE (400 mg/kg) containing capsaicin in sheep concentrate in the middle of lactation (after the peak of lactation) minimized the reduction in milk production during the experiment and improved the quality of the milk, as well as stimulated an antioxidant response systemic.

Keywords: Pepper extract; Oxidants; Antioxidants; Health status; Nutrition; Lactation.

Resumo

Extrato de pimenta (PE, 5 g capsaicina/kg PE) foi adicionado à ração das ovelhas no período de lactação (Dia 75-93) para manter a produção, melhorar a qualidade do leite, e preservar sua saúde. Os grupos foram: T0, controle, (sem PE); T200 (200 mg de PE/kg de concentrado) e T400 (400 mg de PE/kg de concentrado). A redução na produção de leite (L) foi menor nas ovelhas T400 nos dias 0 a 18 e 14 a 18 do que no grupo T0. A conversão alimentar foi menor nas ovelhas dos grupos T200 e T400 do que no grupo T0. A interação entre o tratamento e o dia para proteína, lactose e sólidos totais no leite foi maior nas ovelhas que consumiram PE no dia 18. As contagens de células somáticas no leite foram mais baixas nas ovelhas T400. Os níveis de proteína total e globulina foram mais elevados no sangue dos animais do grupo T400. Houve menores níveis de espécies reativas de oxigênio e lipoperoxidação no soro e leite dos animais dos grupos T200 e T400. No 18º dia, o soro de ovelhas que consumiram PE apresentou maiores níveis de tióis não protéicos e atividades da superóxido dismutase. A inclusão de PE (400 mg/kg) contendo capsaicina no concentrado de ovinos no meio da lactação (após o pico da lactação) minimizou a redução da produção de leite durante o experimento e melhorou a qualidade do leite, bem como estimulou uma resposta antioxidante sistêmica.

Palavras-chave: Extrato de pimenta; Oxidantes; Antioxidantes; Estado de saúde; Nutrição; Lactação.

Resumen

Se añadió extracto de pimienta (PE, 5 g de capsaicina/kg de PE) al alimento de las ovejas durante el período de lactancia (día 75-93) para mantener la producción, mejorar la calidad de la leche y preservar su salud. Los grupos fueron: T0, control, (sin PE); T200 (200 mg PE/kg concentrado) y T400 (400 mg PE/kg concentrado). La reducción en la producción de leche (L) fue menor en las ovejas T400 en los días 0 a 18 y 14 a 18 que en el grupo T0. La conversión alimenticia fue menor en ovejas en los grupos T200 y T400 que en el grupo T0. La interacción entre el tratamiento y el día para proteínas, lactosa y sólidos totales en la leche

fue mayor en las ovejas que consumieron PE el día 18. Los recuentos de células somáticas en la leche fueron menores en las ovejas T400. Los niveles de proteína total y globulina fueron más altos en la sangre de los animales del grupo T400. Hubo niveles más bajos de especies reactivas de oxígeno y lipoperoxidación en el suero y la leche de los animales en los grupos T200 y T400. El día 18, el suero de las ovejas que consumieron PE mostró niveles más altos de tioles no proteicos y actividades de superóxido dismutasa. La inclusión de PE (400 mg/kg) que contiene capsaicina en concentrado de oveja en la mitad de la lactancia (después del pico de lactancia) minimizó la reducción en la producción de leche durante el experimento y mejoró la calidad de la leche, además de estimular una respuesta antioxidante. sistémico.

Palabras clave: Extracto de pimienta; Oxidantes; Antioxidantes; Estado de salud; Nutrición; Lactancia.

1. Introduction

Sheep milk production tends to be impaired in seasons of the year when critical temperatures cause thermal discomfort. This, combined with the drop in the quality of the feed supplied at the end of the lactation peak, can result in large economic losses (Neiva et al., 2004). Persistence of lactation is defined as the ability to maintain production after reaching peak lactation, which occurs between the third and fourth week postpartum (Cannas et al., 2002). Improving the digestion of ruminants has been the focus of research aimed at providing maximum use of the nutrients ingested; the use of active compounds present in plants has garnered substantial attention (Cardozo et al., 2006; Castillo et al., 2012). Modifiers of ruminal fermentation such as capsaicin act by modulating the ruminal microbiota through the inhibition of the growth of some microorganisms and the processing of others; capsaicin decreases the production of acetate and the concentration of ammonia, as well as increasing the production of propionate and the total production of volatile fatty acids (Calsamiglia et al, 2007). According to this researcher, these changes include more acidic pH; therefore, animals that receive feed with higher levels of concentrate are able to make better use of feed (Calsamiglia et al, 2007).

Capsaicin is a bioactive compound found in peppers of the genus *Capsicum* spp., known for its pungent odor. Its chemical name is 8-methyl-N-vanillyl-6-nonenamide, $C_{18}H_{27}NO_3$ (Calsamiglia et al., 2007). It is a crystalline, lipophilic, colorless, and odorless alkaloid (Reyes-Escogido et al., 2011). Among its pharmacological effects, bactericidal and fungicidal activities are particularly important (Zhao et al., 2020). It also has anti-

inflammatory properties capable of reducing the expression of interleukins (Choi et al., 2011), as well as analgesic properties that relieve chronic muscle, joint, and neuronal pains that are not responsive to medication (Reyes-Escogido et al., 2011). Capsaicin can be used topically as an ointment for animals and humans as a treatment for peripheral neuropathies, minimizing lameness, and increasing quality of life (Seino et al., 2003, Roberts et al., 2011). Antioxidant properties are also described in studies with capsaicin because they contain phenolic compounds, in addition to the fact that red peppers have a greater antioxidant capacity than do green peppers (Materska & Perucka, 2005). Recently, a study conducted by An et al. (2020) revealed that the addition of a blend based on the oleoresin of *Capsicum* spp. and eugenol improved performance, digestibility of nutrients, immune responses, and antioxidant capacity of sheep, leading us to hypothesize that this feed additive may have positive effects on production, milk quality, and health status of lactating sheep.

In theory, the effect of capsaicin can cause greater water intake; this effect has been the subject of study in ruminants (Rodriguez-Prado et al., 2012). Water is an essential factor for maintaining the basic functions of the organism, in addition to being closely associated with the animal's zootechnical performance (Nunes, et al., 2011). Studies suggest that the addition of capsaicin to animal feed increases water consumption and can be used to increase production rates in times of low water intake (Zafra et al., 2003; Rodríguez-Prado et al., 2012). Therefore, the objective of the present study was to determine whether adding capsaicin-rich pepper extract to the feed of lactating sheep (mid-lactation period) would maintain milk production and improve milk quality, as well as preserving animal health.

2. Materials and Methods

This scientific research took place through the quantitative experimental method, according to Fonseca (2002), Gil (2008) e Gerhard & Silveira (2009).

2.1 Pepper extract

The feed additive used in this study is a commercial product based on pepper extract (Capsin®; Nutriquest). The chemical composition of the pepper extract was analyzed according to AOAC (2000), being detected the concentration of dry matter (920 g/kg), ether extract (444 g/kg), crude protein (64.4 g/kg), detergent fiber neutral (293 g/kg), and acid

detergent fiber (229 g/kg). The quantification of capsaicin in the pepper extract was performed using gas chromatography and revealed a concentration of 5.0 g/kg.

2.2 Animals and experimental design

Thirty multiparous Lacaune ewes (third order of lactation), mid-lactation period (75 to 93 days postpartum) and with an average body weight of 68 ± 3.8 kg were used in this experiment, which was carried out at the end of autumn on a farm located in Chapecó, Santa Catarina, Brazil.

The animals were housed in an open shed on a beaten floor covered with wood shavings, separated by group in three stalls (24 m^2). The groups consisted of ten animals each and were identified as follows: the T0 – control group (without extract), the T200 treatment group (200 mg PE/kg concentrate), and the T400 treatment group (400 mg PE/kg concentrate).

The concentrate (1.2 kg of animal/day) was offered in equal proportions in the morning and afternoon (8:00 AM and 5:00 PM) throughout the experimental period. To guarantee the concentrate intake, animals were restrained in headlocks. The concentrate was produced from soybean meal, ground corn, and a vitamin and mineral complex.

After concentrate was ingested (100%/ewes/day), with the animals remaining restrained in headlocks, corn silage (3.8 kg animal/day) was offered in the morning and afternoon. It remained for 30 min until the ingestion of most available silage had occurred (between 80 to 90%); then, the sheep were released from the pen and stayed in the collective stall. Afternoon (1:00 PM), the sheep had Tifton chopped hay available in a individual feeder (0.2 kg/animal/day); therefore, the animals were in collective pens, but the feeding was individualized. Water was offered ad libitum.

There was an adaptation period of 13 days (days 1 to 13) to the environment and feed, an adaptation period major that used by our group research (Jaguezeski et al., 2018; Alba et al., 2019, Santos et al., 2019). Between days 14 and 18 of experiment, the intakes of hay, corn silage, and concentrate were measured individually. The leftover food for the day was collected and weighed in order to measure daily intake for five days consecutively.

2.3 Diet analysis

During the experimental period at 1 to 18 (beginning (day 1), middle (day 9), and end (day 18)), samples of silage and concentrate were collected, identified, and stored frozen

(–20 ºC). On the day of analysis, the three samples taken during the silage and concentrate experiment for each treatment and were homogenized, forming a single sample used for the processes described below.

2.3.1 Bromatological

First, grinding was carried out; feed was concentrated using a hammer-type mill (grain size 1 mm). For forage, we used a knife-type mill (Silva & Queiroz 2002). The feed samples were analyzed according to the AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ethereal extract (EE), method 920.39, and ash, method 942.05. The concentrations of neutral detergent fiber (NDF) and acid (ADF) were measured according to the methodology of Van Soest et al. (1991; without the addition of sodium sulfite). Results are presented in Table 1.

2.3.2 Determination of total phenolic compounds (TPC) and antioxidant activity by elimination of radicals by DPPH

For quantification of total phenolic compounds (TPC) and antioxidant activity by elimination of radicals by DPPH (IC_{50}), we used the methodology described in detail by Alba et al. (2019). For extraction, 0.5 g of samples (concentrate) were dissolved in 50 ml of distilled water. The mixture was placed in an ultrasonic bath (70 W) for 3 h and remained in the dark for more 3 h. Quantification of TPC was performed using the Folin-Ciocalteu colorimetric method and free radical scavenging activity in the extracts was determined as the antioxidant reduction capacity of DPPH radical. All tests were performed in triplicate (Table 1).

Table 1. Ingredients and chemical composition of ingredients and experimental diets.

Ingredients	As fed (kg/day)		Dry matter (DM; kg/day)		
Corn silage (kg)	3.80		1.24		
Concentrate (kg)	1.20		1.06		
Hay (kg)	0.20		0.17		
Total (kg)	5.20		2.47		
Chemical composition	Corn silage	Hay	Concentrate (T0)	Concentrate (T200)	Concentrate (T400)
DM, g/kg	327.4	874	885.8	885.2	889.0
Ash, g/kg DM	42.8	34.4	56.3	58.4	57.8
CP, g/kg DM	85.2	99.7	179.5	179.8	179.9
NDF, g/kg DM	343.7	609	89.0	88.2	90.0
ADF, g/kg DM	175.1	217	34.0	35.8	36.0
EE, g/kg DM	47.0	11.7	42.0	42.3	42.0
CFT (mg EGA/100 g DM)	-	-	0.133	0.145	0.158
IC_{50} (mg/mL)	-	-	3.07	2.33	1.54

Ingredients present in 100 kg of concentrate: corn (70%), soybean meal (25%) and buffering lactation nucleus (5%), i.e., ground corn (671 g/kg), soybean meal (277 g/kg), calcitic limestone (10 g/kg), sodium bicarbonate (4 g/kg) and 37 g/kg of premix (calcium min. 180 max. 220 g; phosphorus min. 32 g; sodium min. 40 g; sulfur min. 20 g; magnesium min. 20 g; cobalt min. 16 mg; iodine min. 17 mg; manganese min. 420 mg; selenium min. 730 mg; zinc min. 730 mg; fluorine max. 600 mg; niacin min. 500 mg; vitamin A min. 95000 IU; vitamin D min. 20000 IU; vitamin E min. 350 IU; monensin sodium 1200 mg; *Saccharomyces cerevisiae* 2.1 x 10^{10} CFU).
[2]Note: DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber) and EE (ethereal extract).
[3]Note: Total phenolic compounds (TPC: mg EGA/100 g DM); Antioxidant activity against DPPH - IC_{50} radical (mg/mL). Source: Authors.

2.4 Measurement of milk production

The milk produced by the animals was measured at the beginning (day 0) and end (day 14) of the adaptation period, and in the experimental period (days 15, 16, 17 and 18) using a "Milk Meter" True Test® meter, Auckland, New Zealand. The average production of the days on which the experiment occurred was presented. The total production was the result of the sum of the two daily milkings (morning and afternoon). Milk production data from days 0, 14 and 18 were used to calculate the percentage of reduction in milk production during the 18-day experiment in the mid-lactation period. Based on data on milk production and feed consumption, feed conversion was calculated.

2.5 Sample collection

Blood was collected after 12 h of fasting. Blood samples for hematological and biochemical analyses were collected on the first day, after the adaptation period and at the end

of the experimental period, on days 0, 14, and 18. Collections were performed with animals fasting for 12 hours of solids, with animals being restrained manually (holding them by the head and flank). Venipuncture from the jugular vein drew blood in vacutainers fitted with specific needles. The tubes used were as follows: with EDTA for blood count and blood smear; and with clot activator (silica) for serum biochemistry and oxidants/antioxidants. To collect serum samples from the clot activator, centrifugation was performed at 5,000 rpm for 10 min.

The serum was pipetted into Eppendorf tubes and frozen (–20 ºC) for further analysis. Milk samples were collected on days 1, 14, and 18, as a final product of complete and homogeneous milking, using "Milk Meter" meters (Tru Test®). All samples were transported to the laboratory in isothermal boxes with ice at –4 ºC.

2.6 Milk analysis

2.6.1 Centesimal composition of milk

The lactose, protein, fat, and total solid concentrations of the milk samples were measured using the LactoStar automatic infrared analyzer, Funke Gerber®, standardized methodology for sheep milk (Alba et al., 2019). Analyses were performed in duplicate.

2.6.2 Somatic cell counts in milk

Somatic cell counts (SCC) present in the milk was performed using a semi-automatic counter (Ekomilk Scan Somatic Cells, Analyzer®). Analyses were performed in duplicate.

2.6.3 Oxidants and antioxidants in milk

Milk samples were used for the analyses. First, protein concentrations in milk samples were measured, and based on this information, the samples were prepared for the analyses described below, according to the specific methodologies for each technique.

The enzymatic activity of superoxide dismutase (SOD) was measured using the method of Beutler (1984), with auto-oxidation in pyrogallol, read by spectrophotometer at 480 nm every 10 seconds for 2 minutes. The results were expressed in U SOD/mg protein.

For the determination of oxygen reactive species (ROS) 10 µL of milk sample, with 12 µL of dichlorofluorescein were incubated at 37 °C for 1 h, without incidence of light (Ali et al., 1992). Subsequently, 488 nm was used for excitation and 520 nm for emission to determine fluorescence and the results were expressed in U DCF/mL.

The determination of lipid peroxidation (LPO) was according to the method of Monserrat et al. (2003) and the results are expressed in µmol CHP/mL.

Analysis of antioxidant capacity against peroxyl radicals (ACAP) was performed according to Amado et al. (2009) and the results were expressed in FU/mg protein.

2.7 Blood analysis

2.7.1 Hemogram

Red blood cell count (RBC), total leukocyte count (WBC) and hemoglobin concentration were performed using a semi-automatic analyzer (CC-530 CELM). The leukocyte differential was performed by means of blood smears on glass slides stained with its own commercial kit (*Panótico Rápido*, Laborclin), cell identification was performed using an optical microscope (100x). Hematocrit was obtained after capillary centrifugation at 10,000 rpm for 5 min (Feldman et al., 2000).

2.7.2 Serum biochemistries

In serum samples, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) were measured, as were levels of total proteins (TP), albumin, glucose, cholesterol, triglycerides and urea using specific commercial kits (Analisa®, Gold Analisa Diagnóstica, Belo Horizonte, Brazil) and semi-automatic equipment (BioPlus 2000®). Subtracting albumin from total proteins, globulin levels were calculated.

2.7.3 *Oxidants and antioxidants in serum*

The variables LPO, ROS, and SOD were also measured in serum, using the same methodology described above for milk samples (section 2.6.3). Non-protein thiols (NPSH)

were measured in serum, according to the methodology described by Sedlak & Lindsay (1968).

2.8 Animal behavior

Animal behavior was evaluated in the morning, right after the individual feeding. As soon as the sheep were released from the headlocks, behavioral analysis continued for a constant period of 90 minutes. The animals were numbered with purple spray (lateral and back-sacral) to facilitate observation. Three trained observers were responsible for data collection for three consecutive days at the end of the adaptation period (days 11, 12 and 13 of the experiment). The observers rotated among groups between days and treatments, ensuring that all observers have assessed the three groups of sheep. An ethogram predicted the observation of frequency and length of stay, consumption of water, hay and silage, as well as leisure time.

2.9 Statistical analysis

The animal was considered the experimental unit for all analyses. All dependent variables were tested for normality using Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and all were normal distributed. Then, all data were analyzed using the MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Lactation efficiency, feed intake, feed conversion, and behavior variables were tested for fixed effect of treatment using animal (treatment) as random effects. All other variables were analyzed as repeated measures and were tested for fixed effects of treatment, day, and treatment × day, using animal (treatment) as random variables and animal (treatment) as subjects. All results obtained on d 0 for each variable were included as covariates in each respective analysis; however, they were removed from the model when $P > 0.10$. The covariance structures were selected according to the lowest Akaike information criterion. The compound symmetric covariance structure was selected for milk concentration of SOD and ROS. The Toeplitz covariance structure was selected for hematocrit, and eosinophils and the first order autoregressive covariance structure were selected for all other variables. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$.

3 Results

3.1 Milk performance, composition and quality

The results of milk performance, composition and quality are presented in Table 2. Effects of treatment × day and treatment were not detected for milk production or concentration of fat. The reduction in milk production (L) was less in the T400 ewes at days 0 to 18 and days 14 to 18 than in the T0 ewes. Effects of treatment were not detected for feed intake, but were detected (P = 0.01) for feed conversion, and T200 and T400 ewes had greater feed conversion than did T0 ewes. There was a significant (P = 0.01) interaction between treatment and day for protein and ($P \leq 0.05$) for lactose; and T400 ewes had greater concentrations of these variables only on d18 compared to T0 and T200 ewes. Effects of treatment × day (P = 0.02) were detected for concentrations of total solids. T200 and T400 ewes had greater milk concentrations of total solid on d 14 than did T0 ewes, and only T400 ewes had greater concentrations on d 18 than did T0 ewes. Effects of treatment × day and treatment were detected ($P \leq 0.04$) for SCC. T400 ewes had lower counts only on d 18, compared to T0 and T200 ewes.

The results of milk levels of oxidant/antioxidants are presented in Figure 1. Effects of treatment × day and treatment were not detected for milk concentration of SOD. However, effects of treatment × day to be detected (P = 0.05) and effects of treatment were not detected (P = 0.43) for milk concentration of ROS, and T400 ewes had lower concentrations only on d 14 than did T0 and T200 ewes. Effects of treatment × day (P = 0.02) were detected for levels of ACAP, and T200 and T400 had greater levels only on d 18 than did T0 ewes. Effects of treatment × day (P = 0.05) were detected for levels of LPO, and T200 ewes had lower levels only on d 14 than did T0 ewes. T400 ewes had lower levels only on d 14, compared to the others.

Table 2. Milk production and composition of Lacaune ewes supplemented with pepper extract.

Variables[1]	Treatments[2]			SEM	*P*-value	
	T0	T200	T400		Treatment	Treatment × day
Production (L)					0.66	0.27
d 0	2.40	2.37	2.38	0.12		
d 14	2.01	2.01	2.04	0.12		
d 18	1.81	1.87	1.97	0.12		

Mean[3]	1.91	1.94	2.00	0.12		
Reduction in milk production (%)						
d 0 to 14	16.2	15.5	14.9	0.08	0.56	
d 0 to 18	24.5[a]	21.5[ab]	17.9[b]	0.09	0.01	
d 14 to 18	9.95[a]	7.14[a]	3.60[b]	0.05	0.01	
Sum of milk production (L)						
d 14-18	9.15	9.25	9.55	0.19	0.54	
Feed intake						
d 14 to 18	79.9	73.1	77.0	13.33	0.43	
Feed conversion						
d 14 to 18	2.19[a]	2.06[b]	2.08[b]	0.10	0.01	
Milk composition						
Protein (g/kg)					0.05	0.01
d 0	3.92	3.99	3.80	0.08		
d 14	3.65	3.84	3.92	0.08		
d 18	3.75[b]	3.87[b]	4.25[a]	0.08		
Mean[3]	3.70B	3.85AB	4.08A	0.07		
Fat (g/kg)					0.62	0.47
d 0	5.24	5.32	5.31	0.19		
d 14	5.48	5.69	5.70			
d 18	5.44	5.76	5.66			
Mean[3]	5.46	5.73	5.68	0.21		
Lactose (g/kg)					0.05	0.01
d 0	5.75	5.90	5.56	0.14		
d 14	5.40	5.64	5.76	0.14		
d 18	5.48[b]	5.69[b]	6.25[a]	0.14		
Mean[3]	5.44B	5.66B	6.00A	0.15		
Total solids (g/kg)					0.11	0.02
d 0	15.29	15.44	14.43	0.38		
d 14	13.80[b]	15.36[a]	15.58[a]	0.41		
d 18	15.48[b]	15.87[ab]	16.75[a]	0.38		
Mean[3]	14.64	15.61	16.16	0.39		
SCC (x 10^3 /mL)					0.01	0.04
d 0	199.7	192.3	209.8	51.3		
d 14	354.9	248.6	244.2	51.3		
d 18	395.4[a]	399.4[a]	144.5[b]	54.0		
Mean[3]	375.1A	324.0A	194.3B	52.3		

[1]SCC, somatic cell count. [2]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate. Differs ($P \leq 0.05$) between treatments by day showed in same line with different letters ([a-b]) (Treat × day). [3]Mean of days 14 and 18 of the experiment, illustrates the effect of the treatment, with different letters ([A-B]) in same line differing statistically ($P \leq 0.05$). Source: Authors.

3.2 Hemogram

The results of hemogram are presented in Table 3. Effects of treatment × day and treatment were not detected ($P \geq 0.11$) for erythrocyte counts, hematocrits, hemoglobin levels, or for counts of leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils.

3.3 Serum biochemistry

The results of serum biochemistry are presented in Table 3 and 4. Effects of treatment × day and treatment were not detected for serum concentration of glucose, albumin, cholesterol, triglycerides, AST, ALT, or GGT. However, effects of treatment ($P = 0.05$) and interaction (days 14 and 18) were detected for serum concentration of total protein and globulin, and T400 ewes had greater concentrations than did T0 ewes. Effects of treatment × day ($P = 0.04$) were detected for serum concentration of urea, and T400 ewes had greater concentrations only on d 18, compared to T0 ewes.

Table 3. Hematological and biochemistry variables of Lacaune ewes supplemented with pepper extract.

Variables	Treatments[1]			SEM	*P*-value	
	T0	T200	T400		Treat	Treat × day
Hematology						
Erythrocytes (x 10^6 µL)	7.94	7.91	7.84	0.33	0.98	0.83
Hematocrit (%)	31.85	30.69	31.17	0.89	0.62	0.82
Hemoglobin (g/dL)	9.66	9.30	9.36	0.23	0.52	0.45
Leukocytes (x 10^3 µL)	11.13	11.78	12.72	1.69	0.76	0.65
Neutrophils (x 10^3 µL)	4.24	4.93	5.23	0.59	0.37	0.51
Lymphocytes (x 10^3 µL)	5.43	5.63	5.73	0.93	0.96	0.75
Monocytes (x 10^3 µL)	0.04	0.05	0.03	0.01	0.61	0.12
Basophils (x 10^3 µL)	0.01	0.02	0.06	0.02	0.10	0.11
Eosinophils (x 10^3 µL)	1.34	1.20	1.66	0.31	0.51	0.13
Biochemistry						
Glucose (mg/dL)	60.28	63.39	60.56	2.43	0.62	0.93
Albumin (g/dL)	3.71	3.81	3.68	0.12	0.73	0.98
Cholesterol (mg/dL)	75.18	82.03	77.36	3.70	0.42	0.66
Triglycerides (mg/dL)	26.42	26.31	24.77	1.07	0.47	0.29
AST (U/L)	123.97	114.30	117.60	7.80	0.68	0.63
ALT (U/L)	20.63	18.53	20.03	0.74	0.14	0.16
GGT (U/L)	109.35	106.31	111.97	6.27	0.81	0.21

[1]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate. Note: There was no statistical difference (P>0.05) between treatments, as well as there was no interaction between treatment versus

day for the variables presented in this table. We present the treatment average considering the 14th and 18th days of the experiment, with the "day 0" being used only as a covariate. OBS: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT).

Table 4. Protein response (total protein, globulin, and urea) of Lacaune ewes supplemented with pepper extract.

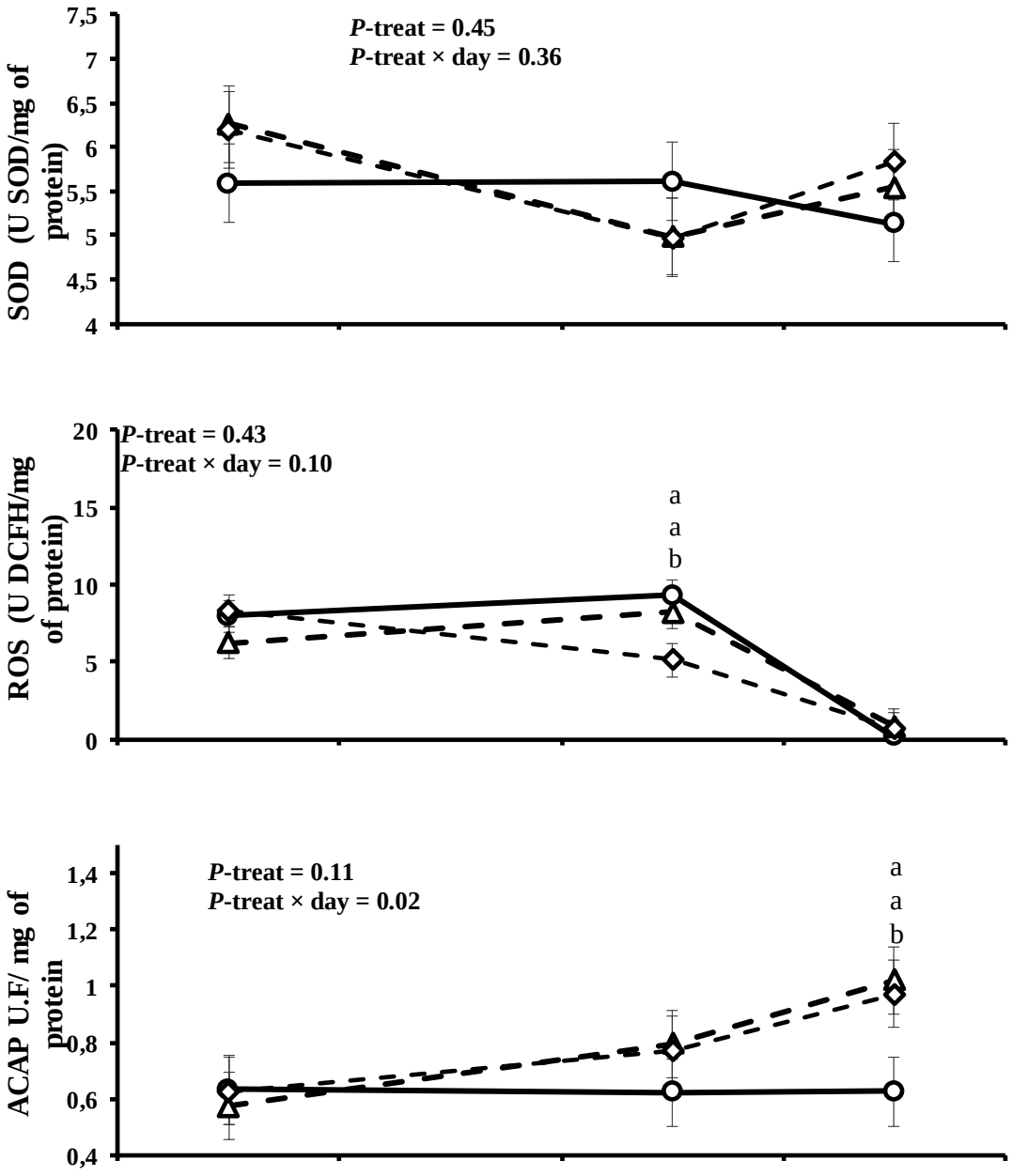
Variables[1]	Treatments[2]			SEM	P-value	
	T0	T200	T400		Treatment	Treatment × day
Total Protein (g/dL)					0.03	0.05
d 0	8.54	8.35	9.54	0.22		
d 14	8.22^{b}	8.94^{ab}	8.97^{a}	0.21		
d 18	8.60^{b}	8.90^{ab}	10.3^{a}	0.22		
Mean[1]	8.41B	8.92AB	9.63A	0.20		
Globulin (g/dL)					0.05	0.04
d 0	4.83	4.60	5.96	0.17		
d 14	4.62^{b}	5.08^{ab}	5.39^{a}	0.18		
d 18	4.74^{b}	5.00^{b}	6.53^{a}	0.19		
Mean[1]	4.68B	5.04B	5.96A	0.19		
Urea (mg/dL)					0.11	0.04
d 0	42.9	37.3	37.5	3.11		
d 14	36.6	43.4	39.1	3.11		
d 18	39.4^{b}	45.3^{ab}	49.2^{a}	3.12		
Mean[1]	38.0	44.3	44.1	3.11		

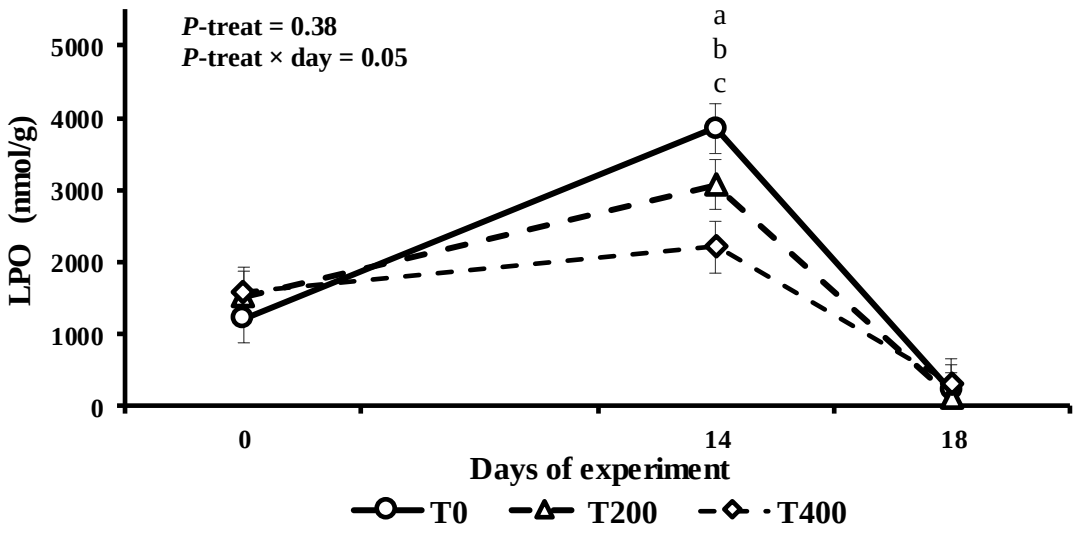
[2]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate. Differs ($P \leq 0.05$) between treatments by day showed in same line with different letters ($^{a-b}$) (Treat × day). [3]Mean of days 14 and 18 of the experiment, illustrates the effect of the treatment, with different letters ($^{A-B}$) in same line differing statistically ($P \leq 0.05$). Source: Authors.

3.4 Serum oxidant/antioxidant status

The results of serum oxidants/antioxidants variables are presented in Figure 2. Effects of treatment × day to be detected ($P = 0.05$) and were not detected ($P = 0.47$) for serum concentrations of NPSH. T200 and T400 ewes had greater concentrations only on d 18 compared with T0 ewes. Effects of treatment × day and treatment were detected ($P = 0.01$) for serum activity of SOD. T200 and T400 ewes had greater activities on d 14 and 18 than did T0 ewes. However, effects of treatment × day ($P = 0.02$) were detected for serum concentration of ROS. T400 ewes had lower concentrations only on d 14, compared to T0 and T200 ewes. Effects of treatment × day and treatment were detected ($P = 0.01$) for serum levels of LPO. T400 ewes had lower LPO levels on d 14 and 18 than did T0 and T200 ewes.

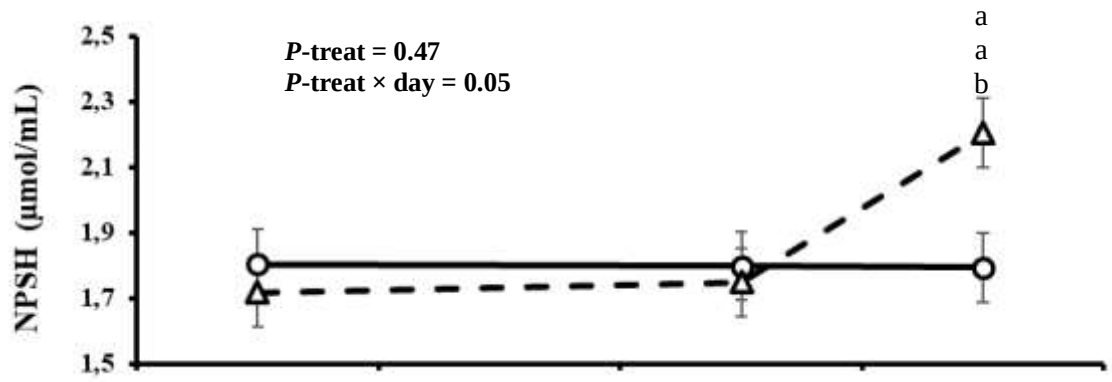
Figure 1. Superoxide dismutase (SOD) activity, and reactive oxygen species (ROS), total antioxidant capacity (ACAP) and lipoperoxidation (LPO) levels in milk of Lacaune ewes supplemented with pepper extract. T0, T200, and T400 represent 0, 200, and 400 mg of pepper extract/kg of concentrate, respectively.

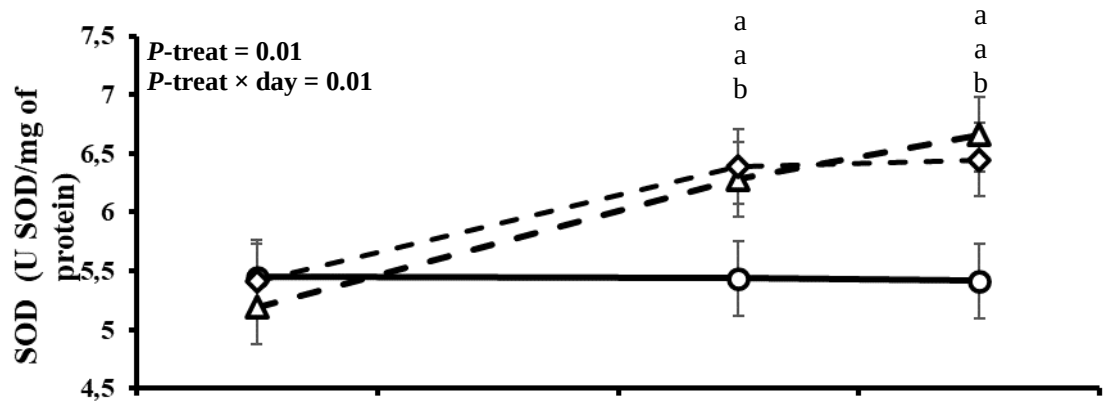


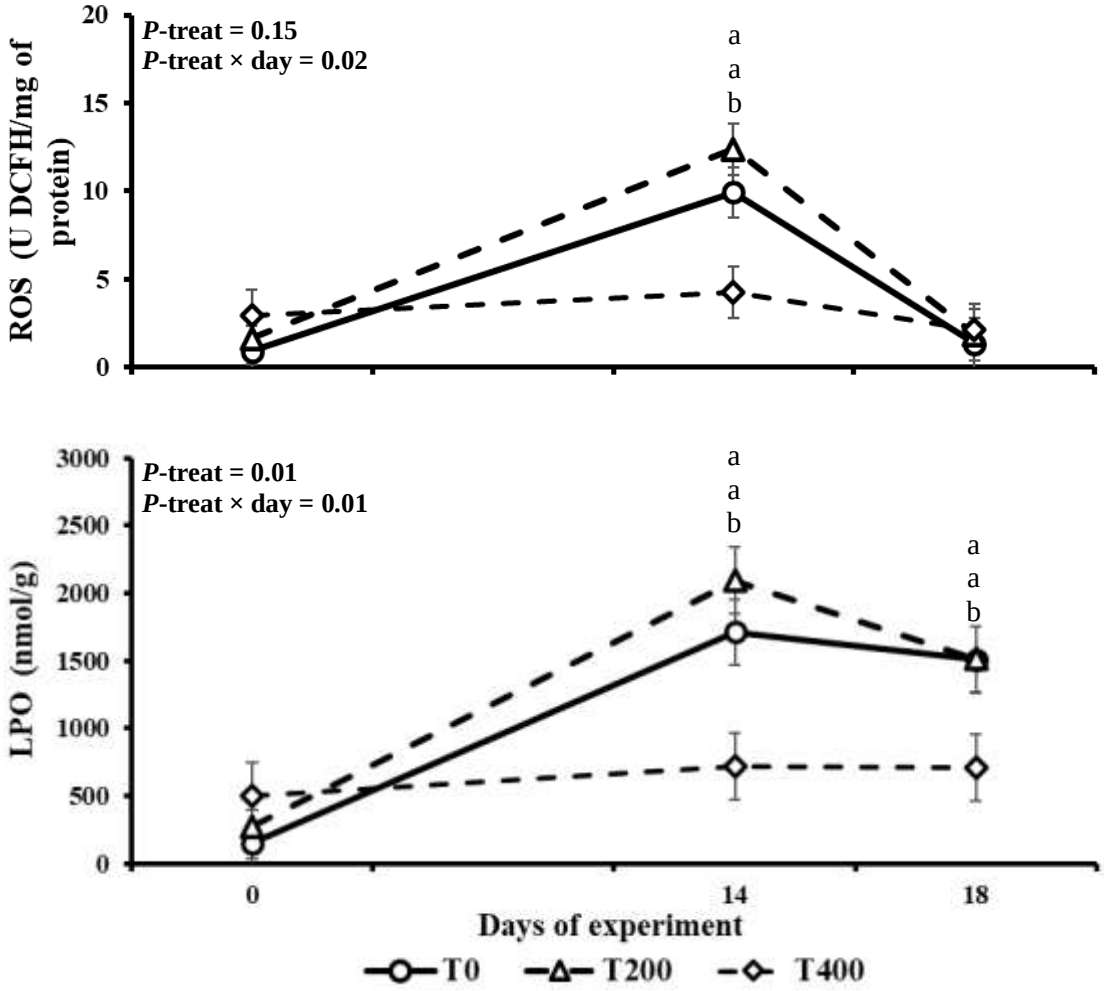


[a-c]Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM. Source: Authors.

Figure 2. Levels of non-protein thiol (NPSH), superoxide dismutase (SOD) activity, reactive oxygen species (ROS) and lipoperoxidation (LPO) levels in serum of Lacaune ewes supplemented with pepper extract. T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate.







[a-b]Differs ($P \leq 0.05$) between treatments each respective day. Vertical bars represent the SEM. Source: Authors.

3.5 Behavior

The results of behavior variables are presented in Table 5. Effects of treatment were not detected ($P = 0.12$) for time spend eating silage and hay or remaining idle as well as the frequency of consuming hay. However, T200 and T400 ewes spent more time drinking water ($P = 0.01$) and had greater frequency ($P = 0.04$) of water drinking. T200 and T400 ewes ($P = 0.05$) to consume silage less frequently.

Table 5. Behavior of Lacaune ewes supplemented with pepper extract.

Variables[1]	Treatments[2]			SEM	P-value
	T0	T200	T400		Treat
Time (min)					
Silage	11.25	7.60	8.25	1.30	0.12
Hay	7.90	9.80	11.30	2.07	0.52
Water	0.80[b]	2.05[a]	1.69[a]	0.36	0.01
Idle	70.05	71.30	69.30	2.69	0.87
Frequency (n°)					
Silage	2.35[a]	1.70[b]	1.60[b]	0.24	0.05
Hay	1.75	1.50	1.60	0.37	0.89
Water	0.70[b]	1.45[a]	1.28[a]	0.21	0.04

[1]Time (minutes when the animals remained in activity, observed for 90 min) and Frequency (times when the animals went to activity, observed for 90 min).
[2]T0, T200 and T400 represents 0, 200 and 400 mg of pepper extract/kg of concentrate.
[a-b]Differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments. Source: Authors.

4. Discussion

The sheep that consumed pepper extract were in lactation phase when milk production started to decrease; however, for the sheep that consumed 400 mg PE/kg of concentrate, this reduction was smaller than that of the control group (T0). In addition, the inclusion of PE in the feed reduced production costs, because the feed conversion of these animals was lower. Furthermore, the milk quality was better when the sheep consumed pepper extract because the milk of these animals had higher concentrations of solids, resulting in lower lipoperoxidation levels and somatic cell counts. Raising solids levels is desirable for sheep milk producers because a large part of contemporary production is destined for production of co-products; and a higher concentration of solids increases production yield. We believe that the lower SCCs were a consequence of lower circulating leukocyte counts; however, this was not confirmed, because there were no differences among groups with respect to these variables. It was not clear to us how the intake of pepper extract reduced SCCs; further studies are needed to investigate the mechanisms involved.

The increase in protein concentration in milk produced by animals that received pepper extract containing capsaicin at the highest dose may be related to the greater availability of amino acids synthesized by the ruminal microbiota, which corroborates the results of a study conducted by An et al. (2020), who found that feed containing 50 mg of a blend containing oleoresin from *Capsicum* spp./kg diet for 15 days improved the digestibility of nutrients, as well as sheep performance. This was also reported in a study with beef cattle that were cannulated and supplemented with *Capsicum* oil, resulting in increased ruminal

concentrations of amino acids, probably due to improvements in the microbial synthesis of proteins deposited in milk and meat (Cardoso et al., 2006).

The animals that received PE with capsaicin cause an increase lactose levels at the end of the experiment. Capsaicin decreased secretion and/or the response to insulin, causing higher blood glucose levels (Van de Wall et al., 2005, 2006). Oh et al. (2017) suggested that capsaicin may increase the availability of glucose to the mammary gland (through its action on insulin secretion), which may explain the greater levels of lactose synthesis. Another effect is the mobilization of fat with reduction in adipose tissue and increased serum levels of free fatty acid. Capsaicin increased the number of defense cells such as neutrophils and lymphocytes (Franco-Penteado et al., 2006; Takano et al., 2007; Oh et al., 2015). For these reasons, we imagine that the increase in globulins was related to this same event, as a result of greater production of immunoglobulins. The administration of *Capsicum* oleoresin to cattle increased the number of eosinophil counts (Oh et al., 2015); instead, we observed an increase in basophil counts. Corroborating this effect on the immune response, An et al. (2020) found that 50 and 80 mg of a blend containing oleoresin from *Capsicum* spp. caused an increase in the production of IgG and IgM; the authors concluded that this caused a stimulating effect on the humoral immune response of sheep supplemented with a blend containing this additive.

The animals that received less capsaicin showed higher water consumption, both in terms of frequency and in terms of time drinking. In similar studies, higher water intake was also observed, followed by even higher feed consumption on the part of animals that received capsaicin (Cardozo et al., 2006; Rodríguez-Prado et al., 2012). Increased water consumption is believed to be related to the pungent properties of the pepper extract. For the production of milk, it is necessary to ingest large amounts of water that pass into the bloodstream and become available to the mammary gland. The addition of capsaicin to cattle feed increased milk production (Oh et al., 2015); we believe that the smallest reduction in milk production in the T400 sheep observed in our experiment may be related to this greater water intake by the animals that received pepper extract, because water consumption positively correlates with milk production.

The concentrate containing pepper extract consumed by the sheep had a greater amount of total phenolic compounds and greater antioxidant activity (Table 1). According to the literature, phenolic compounds such as capsaicin are known for their high antioxidant activity and their ability to protect against the formation of free radicals through the power to reduce their hydroxy groups (Materska & Perucka, 2005; Zhuang et al., 2012). Therefore, as expected, the antioxidant defense responded to the feed, as serum levels of non-protein uncles

and SOD activity were higher in sheep in the groups that received PE; this was probably reflected in higher levels of total antioxidants in milk.

Endogenous antioxidant enzymes such as SOD and CAT are the primary mediators of intracellular defenses against oxidative stress by neutralization of free radicals (Mates et al., 1999). For this reason, the increase in SOD activity we observed is also an indication of better ability to remove superoxide anion, an important free radical (Vinã et al., 2018), possibly explaining the decrease in levels of serum ROS, produced mainly in cellular respiration. In our study, ROS levels were lower in the serum and milk of sheep that consumed PE; this suggests that levels of lipid peroxidation in milk were also lower. An et al. (2020) reported that 50 and 80 mg of a blend containing oleoresin from *Capsicum* spp./kg feed increased non-enzymatic antioxidant activity, as well as stimulating the activity of enzymes such as SOD, CAT, and glutathione peroxidase that have direct effects on oxidants and consequently on the reduction of lipoperoxidation, similar to what was observed in the present study. These results of the antioxidant status of milk when analyzed together with the lower CCS and the higher percentage of solids allow us to conclude that the inclusion of pepper extract improved milk quality.

5. Conclusion

Pepper extract (main 400 mg PE/kg of concentrate) containing capsaicin in the feed of sheep during the mid-lactation period (after the peak of lactation) minimized the reduction in milk production during the experiment and improved feed conversion. The milk produced by these animals that consumed PE had higher levels of protein and lactose, that is, a greater number of total solids, which is desirable for the industrialization and production of derivatives such as yogurt, ice cream, and cheese. There were lower somatic cell counts and levels of lipid peroxidation in milk secondary to the increase in total antioxidants. This suggests that pepper extract may be a potential feed additive that can improve milk quality. Higher levels of globulins indicate that consumption of concentrate with pepper extract stimulated a humoral immune response, combined with increased antioxidant defenses and decreased levels of oxidation in the blood.

We suggest that further studies be carried out with greater challenges so that the already positive results are exacerbated. Studies at different times, in longer periods, where unfavorable environmental conditions and / or low production animals exist. In addition to

differentiated assessments, to elucidate the exact mechanisms of action that caused the results obtained, for example: the ruminal microbiota.

Ethics committee

This experiment was carried out in accordance with animal welfare practices and approved by the Ethics Committee for the Use of Animals in Research (CEUA/UDESC), protocol number 1027070519.

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