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Benefits of the inclusion of açai oil in the diet of dairy sheep in heat stress on health and milk production and quality

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ABSTRACT

The objective of this study was to determine whether the addition of açai (*Euterpe oleracea*) oil in the diets of lactating sheep under heat stress exerted beneficial effects on health as well as milk production and quality. Eighteen multiparous Lacaune sheep (2 or 3 parities; 28–30 days of lactation; average milk production of 1.7 L/sheep/day) were stratified by parity and milk production and were assigned randomly to 1 of 2 treatments (9 sheep/treatment): diet supplemented with 2% of soybean oil (SOY) or 2% of açai oil (AÇAI) in the concentrate for 14 days. The amount of oil added in the diet was equivalent to 0.65% of the total diet (dry matter basis). Blood and milk samples were collected on days 1, 10 and 14. On day 14, the AÇAI group sheep had lower serum concentrations of leukocytes, neutrophils, and lymphocytes than did the SOY group sheep. On day 14, AÇAI group sheep had lower serum concentration of triglycerides and urea, milk concentration of fat and total solid and milk lipid peroxidation than did SOY group sheep. However, on day 14, AÇAI group sheep had higher serum concentrations of glucose and globulin, serum and milk antioxidant capacity against peroxyl radicals, milk production and productive efficiency than did SOY group sheep. The fatty acids profile in milk did not differ between groups. These data suggest that açai oil improved the antioxidant activity in serum and milk and improved milk production and quality in dairy sheep under heat stress.

1. Introduction

The Brazilian sheep herd comprised approximately 18.41 million head in 2016 (IBGE, 2016), making Brazil the eighteenth largest global producer in 2015 (FAO, 2015). The sheep industry represents an important source of income for small and medium-sized farms in Brazil, with signs of growth. The production of cheese from sheep milk has increased in some countries, because it is extensively exported and enjoys high market appreciation (Park and Wendorff, 2006). Compared to other milks, sheep milk is notable for its higher concentrations of solids and nutrients, including calcium, phosphorus, iron, magnesium, potassium, vitamins A, C, D, E as well as B-complex vitamins (Alichanidis and Polychroniadou, 1995; Yuksel et al., 2012).

Fat and oils are important nutrients in animal diets, because they are highly concentrated sources of energy, and are essential components of the functional and physical structures of cells (Cunningham and Klein, 2008). Oil in diets can result in important effects in ruminants, as the production of conjugated linoleic acid, an important anticarcinogenic agent for humans found in the milk (Lin et al., 1995). Açai is consumed by humans because of its high concentration of protein, fibers, lipids, vitamins and minerals, with lipid concentrations reaching 40.75%. Of the total, 52.70% is oleic acid (omega 9) and 25.56% is palmitic acid (Alexandre et al., 2004). Nevertheless, little is known about its use in ruminant nutrition in spite of these added

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values. Therefore, we focused on açai oil (*Euterpe oleracea*), a by-product extracted from the fruit's core.

Açai contains relatively high concentrations of anthocyanin, a component that belongs to the flavonoids family and is responsible for the fruit's color (Pereira, 2008). Anthocyanin has several pharmacological and medicinal properties, including antimicrobial, anti-inflammatory and anti-carcinogenic, that collectively prevent or reduce the oxidation of low-density proteins that are associated with the pathogenesis of neurological disorders and cardiovascular diseases (Kuskoski et al., 2002; Alasalvar et al., 2005). According to Pacheco-Palencia et al. (2008), açai oil contains phenolic acids, catechin and numerous procyanidin oligomers at greater concentrations than are found in açai pulp. Additives and supplements in livestock diets have been seen as tools for transferring biological properties to the final product. For example, a recent study demonstrated that palm oil supplements in sheep diets increased the concentration of unsaturated fatty acids and reduced the saturated fatty acids in milk and yogurt (Bianchi et al., 2017), resulting in benefits to the consumer. The inclusion of a by-product (grape residue flour) with high concentrations of phenolic compounds (such as are found in açai oil) in the diets of dairy sheep under heat stress increased antioxidant and anti-inflammatory activities, improving milk quality and reducing somatic cell count and lipid peroxidation (Alba et al., 2019). Therefore, our hypothesis was that the addition of açai oil in the diet of sheep would increase the anti-inflammatory and antioxidant activity as well as improving milk production and quality. We evaluated whether the addition of açaí oil in the diet of lactating sheep under heat stress exerted beneficial effects on health and milk production and quality.

2. Material and methods

2.1. Oils

Açai and soybean oils were obtained from commercial companies (Açai oil, Gran oils, São Paulo, SP, Brazil and Soya, Bunge Alimentos, Rondonópolis, MT, Brazil, respectively). The concentration of fatty acids from oils was analyzed. About 30 mg of oils samples were used for derivatization in fatty acid methyl esters (FAME) according to Hartman and Lago (1973) with some modifications. First, basic catalysis was performed using KOH in methanol (0.4 mol/L) followed by acid catalysis using H_2SO_4 in methanol (1 mol/L). In both steps, the tubes with the samples were heated at 100 °C for 10 min. Finally, hexane was added to partition the FAME. The FAME was analyzed by gas chromatography with a flame ionization detector (GC-FID; model Star 3600, Varian, USA) by injecting 1 µL into a split/splitless injector with ratio 20:1, heated at 250 °C. The separation occurred in an HP-88 capillary column (100 m × 0.25 mm × 0.20 µm; Agilent Technologies, USA) with an initial temperature of 50 °C for 1 min, increasing to 185 °C (15 °C/min), then to 195 °C (0.5 °C/min) and then to 230 °C (15 °C/min) and remaining at this temperature for 5 min. The carrier gas used was hydrogen with a constant pressure of 35 psi, and the detector was maintained at 250 °C. FAME identification was performed by comparing sample retention times using FAME Mix-37 (P/N 47885-U; Sigma-Aldrich, USA) and the results were expressed as percentage of the total area considering FID correction factors (Visentainer, 2012). Results are shown in Table 1. According to the literature, açaí oil has greater concentrations than soybean oil of phenolic compounds, including vanillic acid, syringic acid, p-hydroxybenzoic acid, protocatechuic acid, ferulic acid, catechin, and several procyanidin oligomers (Siger et al., 2008; Pacheco-Palencia et al., 2008).

2.2. Animals and experimental design

The experiment was conducted at a commercial dairy sheep farm in Chapecó, Santa Catarina, Brazil. We used 18 multiparous (2 or 3 parities) Lacaune sheep (29 ± 1 days in lactation; average milk production of 1.7 L/sheep/day). The animals were stratified by parity and milk production and were assigned randomly to 1 of 2 treatment groups (9 sheep/treatment): diet with 2% soybean oil (SOY) or diet with 2% açai oil (AÇAI) in the concentrate, for 14 days. The oils were included at 2% [dry matter basis (DM)] in the concentrate, equivalent to 0.65% of the total diet (DM), and both oils had similar energetic values, according to the manufacturer's information. In addition to concentrate, we provided corn silage and Tifton hay (*Cynodon spp*. cv.

Table 1
Fatty acid composition of açai and soybean oils.

Fatty acid (%)	Soybean oil	Açai oil
C14:0 (Myristic acid)	0.06	–
C16:0 (Palmitic acid)	14.6	11.3
C16:1 (Palmitoleic acid)	0.05	–
C18:0 (Stearic acid)	3.00	1.81
C18:1n9c (Oleic acid)	30.3	37.4
C18:2n6c (Linoleic acid)	45.5	44.6
C18:3n3 (Linolenic acid)	5.33	3.45
C24:0 (Lignoceric acid)	0.06	–

Table 2
Ingredients, chemical and fatty acids composition of experimental diets.

Ingredients	As fed (Kg/animal/day)		DM (Kg/animal/day)	
Corn silage	3.6		1.22	
Hay	0.25		0.22	
Concentrate[a]	0.80		0.70	
Chemical composition[b]	**Corn silage**	**Hay**	**SOY group[a]**	**AÇAÍ group[a]**
Dry matter, %	33.93	89.2	87.99	89.34
Ashes, % DM	2.85	6.01	7.52	8.15
Crude protein, % DM	7.11	7.27	19.49	19.93
NDF, % DM	41.90	64.45	8.71	11.73
ADF, % DM	23.89	40.14	4.27	6.53
EE, % DM	4.41	1.64	3.98	3.90
Profile of fatty acids in the diet				
Fatty acids (%)[c]	**Corn silage**	**Concentrate SOY group[a]**	**Concentrate AÇAÍ group[a]**	
C14:0	0.20	0.03	0.04	
C16:0	16.05	12.75	12.23	
C16:1	–	0.03	0.05	
C17:0	–	0.04	0.04	
C18:0	1.50	2.30	2.77	
C18:1n9c	29.91	29.48	34.61	
C18:2n6c	45.66	51.67	47.06	
C18:3n3	5.93	2.78	1.94	
C20:0	0.20	0.46	0.51	
C20:1n9	–	0.13	0.20	
C20:2	–	0.00	0.03	
C22:0	0.22	0.21	0.32	
C24:0/C20:5n3	0.32	0.12	0.20	

[a] Ingredients present in 100 kg of concentrate: ground corn (67.13%), soybean meal (27.78%), calcitic limestone (0.93%), sodium bicarbonate (0.46%) and 3.7% mineral mix (calcium 195–220 g; phosphorus min. 39 g; sodium min. 75 g; sulfur min. 18 g; magnesium min. 12 g; cobalt min. 4 5 mg; iodine min. 65 g; manganese min. 1300 mg; selenium min. 15 mg; zinc min. 3500 mg; niacina min. 500 mg; vitamin A min. 316000 mg; vitamin D3 min. 63000 UI; vitamin E min. 650 UI; fluoride max. 390 mg in 1.0 kg of product).

[b] Note: NDF (neutral detergent fiber), ADF (acid detergent fiber), EE (ethereal extract).

[c] Note: Fatty acid in the concentrate of both groups: myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), linolenic acid (C18:3n3), arachidic acid (C20:0), cis-11-eicosenoic acid (C20:1n9), cis-11,14-eicosadienoic (C20:2), behenic acid (C22:0), lignoceric acid (C24:00) and cis-5,8,11,14,17-eicosapentaenoic (C20:5n3).

Tifton 85) to the animals (Table 2). The experiment lasted 14 days, with 10 days of adaptation to the treatments (Jaguezeski et al., 2018). Before the study, the sheep received the same diet but without the inclusion of oils.

On day 1, the sheep were housed in two pens (one pen/treatment; nine sheep/treatment; 24 m^2/pen) in a covered barn with access to water ad libitum and were fed in individual feed bunks. The animals were milked twice a day (0600 h and 1600 h) and after milking, were provided 400 g of concentrate (800 g/day), 1.8 kg of corn silage (3.6. kg/day) and 125 g of hay/sheep (250 g/day), totaling 4.65 kg/day as fed. The feed intake and orts were measured between days 11 and 14. The diets were isoproteic and isoenergetic (Table 1).

The experiment was carried out in the south of Brazil, in a barn without air conditioning, with open sides. The study was conducted in the summer; temperature and relative humidity were measured inside the barn every afternoon (1400 h). During the experiment, the temperature ranged from 29 °C to 38 °C, and the relative humidity ranged from 66% to 77% (Supplementary Fig. 1). The temperature-humidity index (THI) was calculated according with the equation of Mader et al. (2006): THI = 0.8 × ambient temperature + [(% relative humidity ÷ 100) × (ambient temperature − 14.4)] + 46.4. The THI ranged from 80.4 to 93.2. The animals showed clinical signs of heat stress during the hottest times of the day, characterized by rapid breathing, intense salivation, and open mouths, with tongue exposure in many animals.

2.3. Dietary chemical analysis

Feed samples were collected and the concentration of crude protein (CP%), neutral detergent fiber (NDF%), acid detergent fiber (ADF%), ethereal extract (EE%) and ashes were evaluated using near-infrared reflectance (NIR) spectrophotometry (Shenk and Westerhaus, 1994; Shankar, 2015).

2.4. Milk collection

Milk production was individually measured on days 1, 10, 11, 12, 13 and 14, during morning and afternoon milking, using a Milk Meter®. Productive efficiency (%) was calculated individually based on milk production on days 1, 10 and 14 of the experiment and the increase in milk production was calculated from days 1–10 and days 1–14 (using the average of production from day 11–14 for this calculation) each group. The difference in milk production was assigned to the percentage that was then used in the statistical analysis for productive efficiency.

On days 1, 10 and 14, we collected 40 mL of milk from each sheep, placing it in tubes, refrigerated at 4 °C, and subsequently analyzing the chemical composition. Five milliliters of milk from each sheep was allocated in two microtubes and stored at −20 °C for subsequent measurement of lipoperoxidation (LPO), antioxidant capacity against peroxyl radicals (ACAP) [1 mL] and fatty acid profile [4 mL].

2.5. Milk analyses

2.5.1. Chemical composition

In the fresh milk samples, the concentration of fat, protein, and lactose were measured using methodologies defined by IDF Standard on a LactoStar Funke Gerber® infrared analyzer.

2.5.2. Analysis of oxidative/antioxidant status in milk

In frozen milk samples (1 mL), the concentration of protein in milk was determined using the Coomassie Blue method following the methodology described by Read and Northcote (1981) using bovine serum albumin as a standard. The LPO in milk was analyzed following the methodology (FOX method) described by Hermes-Lima et al. (1995) with some modifications by the authors, based on the oxidation of Fe(II) under acidic conditions. The FOX method measures the concentration of lipid peroxides, one of the main products of lipid peroxidation. For LPO measurements, $FeSO_4$ (1 mM), H_2SO_4 (0.25 M), xylenol orange (1 mM, Sigma) and MilliQ water were sequentially added. Samples or methanol (blanks) were added and incubated for 30 min. The absorbance (550 nm) was determined and Cumene hydroperoxide (CHP; Sigma) was used as a standard. Lipid peroxidation (LPO) was expressed as cumene hydroperoxide (CHP) equivalents per nmol per mL of milk. The ACAP was determined according to the method described by Amado et al. (2009) with some modifications for milk. The method consists of finding the antioxidant capacity of tissues using a fluorescent substrate (2′,7′ dichlorofluoresceindiacetate - H_2DCF-DA) and the production of peroxyl radicals by thermal decomposition of 2.2′-azobis (2-methylpropionamidine) dihydrochloride (ABAP). The fluorescence was determined using a microplate reader (Spectramax I3) at 37 °C (excitation: 485 nm; emission: 530 nm) with readings every 5 min, over 30 min. The results were expressed as relative area (difference between the area with and without ABAP divided by the area without ABAP). According to this methodology, a low relative area means high ACAP.

2.5.3. Fatty acids profile in milk and diet

Lipid extraction from milk (4 mL) and diets (4 g) samples was performed according to the method of Bligh and Dyer (1959), where 20 mg of lipids were subjected to methylation as described by Hartman and Lago (1973). First, saponification reaction was carried out (0.4 M of NaOH methanolic solution; at boiling point water bath for 10 min) followed by acid-catalyzed esterification (1 M H_2SO_4 methanolic solution; at boiling point water bath for 10 min). Fatty acid methyl esters (FAME) were partitioned with hexane 2 mL and analyzed on a gas chromatograph equipped with a flame ionization detector (GC-FID, Varian Star 3400CX, Walnut Creek, US). Samples were injected in split/splitless injector (1 μL), operated in split mode at 20:1 ratio at 250 °C. The carrier gas was hydrogen at a constant pressure of 39 psi. The FAMEs were separated on an SP™-2560 capillary column (100 m × 0.25 mm × 0.2 μm; Supelco, Bellefonte, PA, US). The initial column temperature was 140 °C, maintained at this temperature for 5 min, then increased to 180 °C (8 °C/min), then to 210 °C (4 °C/min) and then to 250 °C (20 °C/min), remaining at this temperature for 7 min. The detector was maintained at 250 °C. FAME identification was performed by comparing the retention times of the analyses with the standards (FAME Mix-37, trans-vaccenic methyl ester, docosapentaenoic methyl ester (DPA), a mixture of conjugated of linoleic methyl esters (CLAs) and isomers of linoleic and linolenic methyl esters, all acquired from Sigma-Aldrich (St. Louis, MO, US). The results are expressed as percentage of each fatty acid, identified in the lipid fraction, considering the chain size equivalent factor of FAME for FID and conversion factor of ester to the respective acid (Visentainer and Franco, 2006).

2.6. Blood sample collection

Blood samples were collected immediately before the morning meal on days 0, 10 and 14, via puncture of the jugular vein using tubes containing clot activator (4 mL) and EDTA as anticoagulant (4 mL). The tubes containing EDTA were used to perform the hemogram, while tubes containing clot activator were used to obtain serum after centrifugation (800 rpm during 10 min). The hemogram was analyzed within 24 h after collection, and the serum was stored in microtubes at – 20 °C until analyses.

2.7. Blood analyses

2.7.1. Hemogram

Erythrocyte and total leukocyte counts, as well as hemoglobin (Hb) content were measured using a semi-automated analyzer Celm® 530. Hematocrit was obtained after capillary centrifugation (1000 rpm for 5 min). Leukocyte differential counts were performed using blood smears stained with commercial dye (Romanowsky method) using a light microscope at 1000× magnification.

2.7.2. Serum biochemistry

The serum concentration of albumin, total protein, triglycerides, glucose, cholesterol, and urea were measured on a semi-automated analyzer BioPlus 2000® using commercial kits (Analisa®, Gold Analisa Diagnóstica, Belo Horizonte, Brazil) following the manufacturer's recommendations. Globulin levels were calculated as total protein – albumin.

2.7.3. Oxidant and antioxidant status

In serum, LPO levels were analyzed according to Hermes-Lima et al. (1995) and ACAP levels according to Amado et al. (2009), as described in detail for milk analyses.

2.8. Statistical analysis

Sheep was considered the experimental unit for all analyses and the data were analyzed using R-language, v.2.15.1 (R Development Core Team 2012). The results were tested for normality using the Shapiro-Wilk test. Homogeneity was assessed using the Levene test. Data that did not show normal distribution were transformed to logarithms. Subsequently, the data were analyzed using two-way analyses of variance (ANOVA). The statistical model used was:

$$y_{ijk} = \mu + T_i + D_j + A_k + (T \times D)_{ij} + \varepsilon_{ijk}$$

where: y_{ijk} = ijk observation; μ = overall average; T_i = treatment effect; D_j = day effect; A_k = animal effect; $(T \times D)_{ij}$ = interaction effect between treatment i and day j; ε_{ijk}: random error. The least squares averages were compared using the Tukey test if a significant F-test was detected. Significance was defined as $P \leq 0.05$.

3. Results

3.1. Intake

During the experimental period (day 10–14) the intake by sheep in the SOY and AÇAI groups were 90.3% and 94.5% of the feed offered ($P = 0.836$), respectively. All sheep consumed all the concentrate offered, and the daily orts were only silage and hay.

3.2. Milk production and composition

Milk production was greater for AÇAI groups sheep than for SOY group sheep on day 14 of the experiment ($P < 0.01$). Over time, milk production increased in both groups, reaching a significant difference in the AÇAI group sheep on day 14 ($P \leq 0.001$; Fig. 1). Productive efficiency was significantly greater ($P \leq 0.05$) for AÇAI group sheep from days 1–10, days 10–14 and days 1–14 (Fig. 1). AÇAI group sheep had lower concentrations of milk fat on day 14 of the experiment ($P \leq 0,001$), explaining the lower concentration of milk total solids ($P \leq 0.05$), compared to those of SOY group sheep (Fig. 2). No significant differences were observed between groups in terms of protein and lactose concentrations ($P > 0.05$; Fig. 2).

3.3. Oxidative/antioxidant status in milk

AÇAI group sheep had greater milk ACAP levels ($P \leq 0.05$) and lower LPO levels ($P \leq 0.05$) on day 14 of the experiment (Fig. 3).

3.4. Fatty acids profile in milk

No significant differences were observed for fatty acid profile between groups during the experiment ($P > 0.05$; Tables 3 and 4). Over time, the concentration of butyric, total SFA and total PUFA increased, and the concentration of total MUFA decreased in the milk of both groups ($P \leq 0.05$; Tables 3 and 4).

3.5. Hemogram

No significant differences were observed between groups for total erythrocyte counts, hemoglobin content or hematocrit ($P > 0.05$). Over time, the number of erythrocytes and hematocrit increased in both groups ($P \leq 0.05$). AÇAI group sheep had lower total leukocyte counts ($P \leq 0.05$), and consequently lower number of neutrophils ($P \leq 0.001$) and lymphocytes ($P \leq 0.05$) on day 14, compared to SOY group sheep. No differences were detected for monocyte and eosinophil counts between groups ($P > 0.05$). Over time, the number of total leukocytes and lymphocytes decreased only in AÇAÍ group sheep ($P \leq 0.05$; Table 5).

3.6. Serum biochemistry

AÇAI group sheep had greater ($P \leq 0.05$) serum concentrations of glucose and globulin, and lower ($P \leq 0.05$) serum concentrations of triglycerides and urea on day 14, than did SOY group sheep (Table 6). Significant differences were not observed for serum concentrations of total proteins, albumin, or cholesterol ($P > 0.05$). Over time, serum concentrations of glucose increased ($P \leq 0.05$) only in AÇAI group sheep, while serum concentrations of triglycerides and urea decreased (Table 6).

3.7. Oxidative/antioxidant status in serum

AÇAI group sheep had greater ($P \leq 0.05$) ACAP in serum and milk, and lower ($P \leq 0.05$) LPO in milk on day 14, than did SOY group sheep (Fig. 3). No differences between groups or over time were detected ($P > 0.05$) in terms of serum LPO (Fig. 3).

4. Discussion

The inclusion of açaí oil in sheep diets improved their health and consequently increased milk production and quality; this knowledge will aid the development of the dairy sheep industry. According to Borges (2009), in addition to supplying energy, functional oils develop biological functions; these may have antimicrobial capacity (similar to the ionophores) as well as antioxidant and anti-inflammatory properties (Kuskoski et al., 2002; Alasalvar et al., 2005; Pacheco-Palencia et al., 2008). In the current study, açaí oil may have increased milk production because of alterations in rumen fermentation (antimicrobial effect) and increased propionate production (similar to the ionophores; Yang and Russell, 1993). According to Aschenbach et al. (2010), rumen propionate is converted in glucose via hepatic gluconeogenesis. This explains the greater serum concentration of glucose in AÇAÍ group sheep.

Açaí oil reduced the concentration of serum urea. Changes in serum urea concentrations correlated with the concentration of ammonia in the rumen. Because açai contains anthocyanin, this had an antimicrobial effect (Kuskoski et al., 2002; Alasalvar et al., 2005).

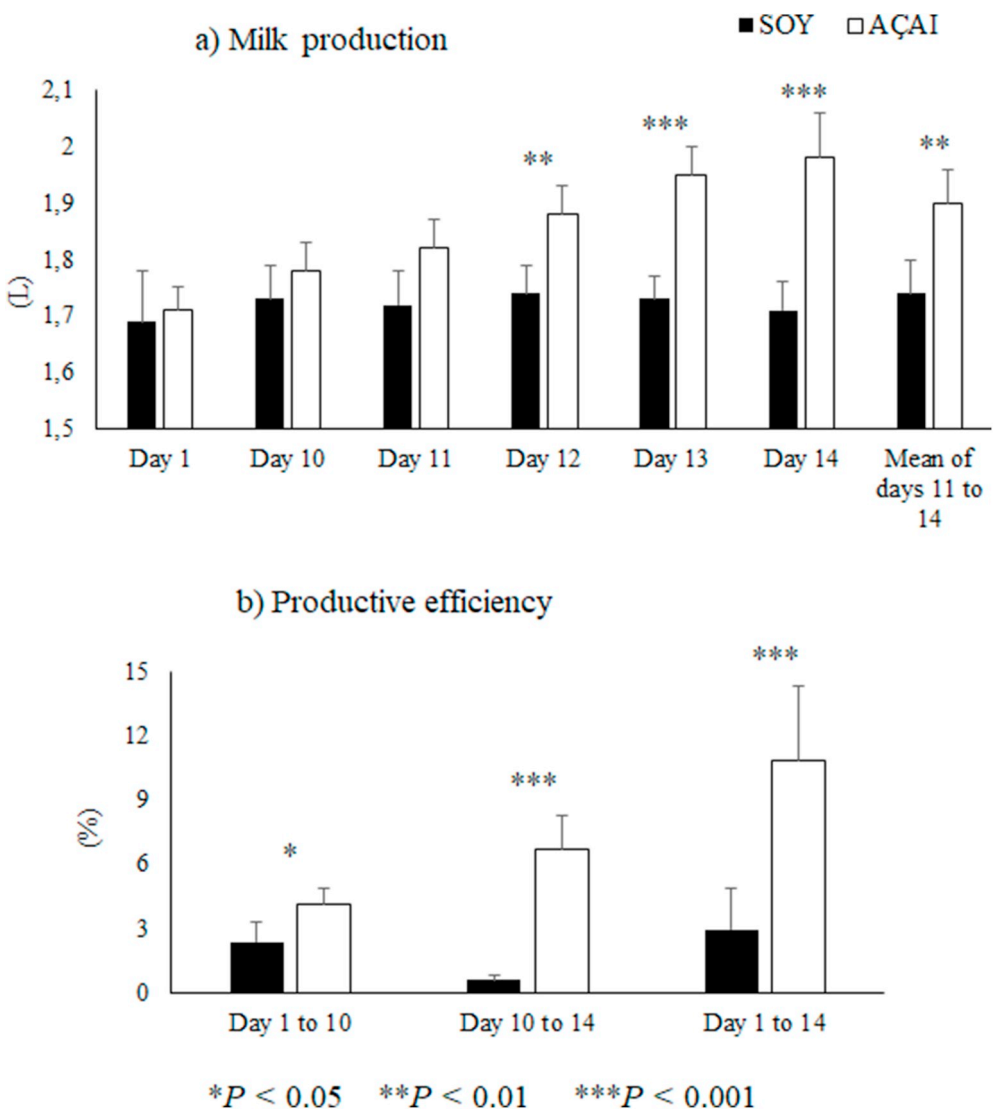

Fig. 1. Milk production from sheep supplemented with açai oil (AÇAI; *Euterpe oleracea*) or soybean oil (SOY) at days 1, 10, 11, 12, 13, and 14. Milk yield was also expressed as mean of the experimental period (day 11–14).

Therefore, açaí oil may have acted in the rumen in a manner similar to that of the ionophores, reducing the concentration of ammonia. This response is associated with reduction in the number of bacteria gram-positive that use amino acids and peptides as energy sources, consequently releasing ammonia in the ruminal environment (Yang and Russell, 1993). This reduction in the use of amino acids and peptides favors their passage into the small intestine with subsequent absorption, increasing the efficiency of the use of nitrogen (Yang and Russell, 1993) and possibly helping to increase milk production, as seen in the current study.

Açaí oil in the diet of sheep reduced the concentration of fat and total solids in the milk. One factor that decreases the concentration of fat in the milk is the high rate of biohydrogenation in diets with high inclusion of unsaturated fatty acids (Hess et al., 2008). This theory is based on the synthesis of trans fatty acids in the rumen, where trans fatty acids inhibit de novo synthesis in the mammary gland (Hess et al., 2008). However, both oils used in our study had a similar fatty acid composition and the rate included in the diet was lower (0.65% of the total diet). Therefore, the reduced concentration of fat in milk was probably not due to the greater biohydrogenation of açaí oil. Fat reduction in the milk of the açaí group was probably was only a consequence of greater milk production, diluting the limited amount of fat produced.

Açaí oil in sheep diets increased ACAP and reduced lipoperoxidation in the milk, as observed in lactating sheep supplemented with others products that stimulate the antioxidant system, including diphenyl diselenide (Biazus et al., 2019) and curcumin (Jaguezeski et al., 2018). The increased concentration of ACAP can be associated with the presence of flavonoids, including anthocyanin and pro-anthocyanin (Cedrim et al., 2018), other phenolic acids, catechin and procyanidin oligomers (Pacheco-Palencia et al., 2008), all of which are important sources of defenses against oxidative stress (Schauss et al., 2006). Supplementation with 2% açai pulp in rat diets improved levels of biomarkers of oxidative stress (Souza et al., 2010), increased serum concentrations of glutathione and reduced lipid peroxidation, minimizing the oxidative stress in diabetic rats (Guerra et al., 2011). Antioxidants in diets decreased somatic cell counts (SCCs) in milk (Colakoglu et al., 2017), improving udder health. This can result in better milk quality for the consumer. We did not analyze SCCs in sheep milk because of technical problems with equipment during the

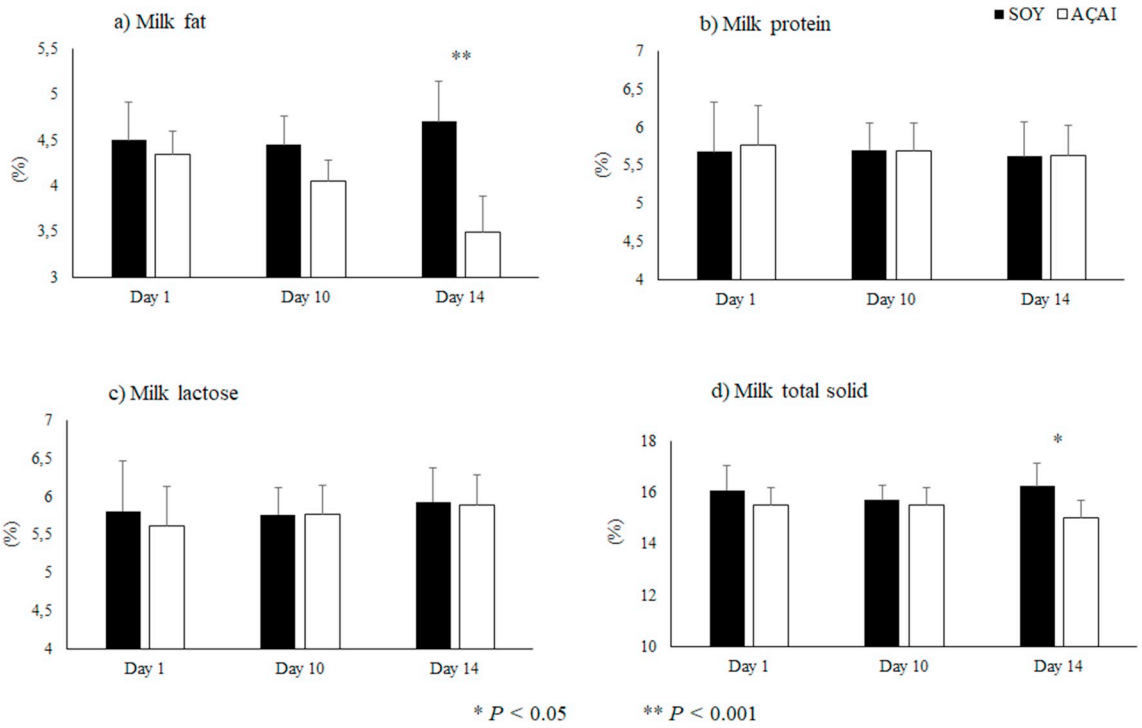

Fig. 2. Milk composition from sheep supplemented with açaí oil (AÇAI; *Euterpe oleracea*) or soybean oil (SOY) at days 1, 10 and 14.

experiment; nevertheless, the increased antioxidant levels in the milk and reduction of serum leukocytes could lead to lower SCCs.

Animals are often exposed to a substantial number of challenges; among them are infectious agents that cause inflammatory process characterized by increased leukocyte numbers, thereby increasing energy expenditure and negatively affecting production. The use of additives capable of minimizing inflammation and reducing circulating and tissue numbers of neutrophils and lymphocytes may improve productive efficiency (Alba et al., 2019). In this study, we showed that açai oil reduced the concentration of total leukocytes attributable to reduced numbers of neutrophils and lymphocytes; this effect probably occurred because of the anti-inflammatory properties of açaí, with positive effects on production. According to Favacho et al. (2011), lipids in açai are involved in the reduction of inflammatory processes, principally involving unsaturated fatty acids such as oleic acid and palmitic acid. Furthermore, açai is a potent inhibitor of cyclooxygenase (COX-1 and COX-2) and lipopolysaccharide (LPS), inductors of nitric oxide formation that results in cytotoxic activity (Rodriguez-Sanoa et al., 1999;

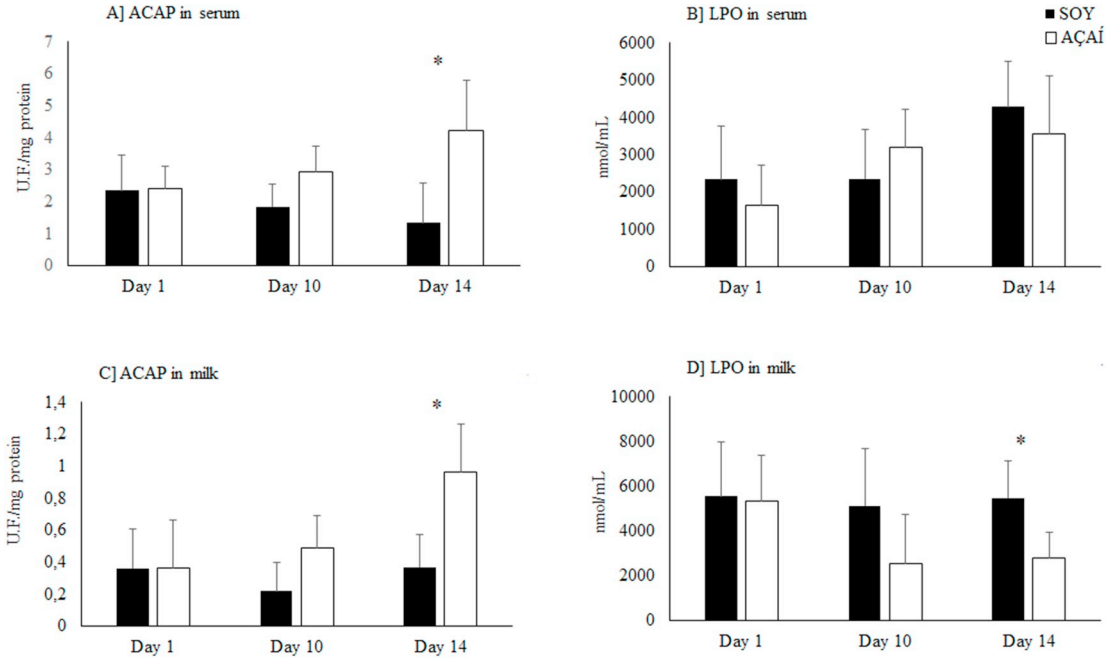

Fig. 3. Antioxidant activity against peroxyl radicals (ACAP) and lipid peroxidation (LPO) in serum and milk of sheep supplemented with açaí oil (AÇAÍ; *Euterpe oleracea*) and soybean oil (SOY).

Table 3
Saturated fatty acids profile in the milk of sheep supplemented with açai oil (AÇAI; *Euterpe oleracea*) or soybean oil (SOY).

Variables	Day	SOY	AÇAI	P-values
C4:0 (Butryic) [a]	1	1.27[b]	1.91[b]	**0.171**
	10	1.17[b]	3.13[a]	**0.065**
	14	3.01[a]	3.28[a]	**0.841**
C6:0 (Caproic)	1	1.34	1.66	**0.337**
	10	1.45	1.55	**0.498**
	14	1.69	1.67	**0.847**
C8:0 (Caprylic)	1	1.35	1.87	**0.397**
	10	1.63	1.74	**0.405**
	14	1.97	1.94	**0.910**
C10:0 (Capric)	1	5.37	7.32	**0.098**
	10	7.09	6.88	**0.590**
	14	8.23	7.66	**0.809**
C11:0 (Undecanoic)	1	0.10	0.00	**0.789**
	10	0.15	0.16	**0.951**
	14	0.22	0.23	**0.947**
C12:0 (Lauric)	1	3.08	4.25	**0.174**
	10	4.15	3.94	**0.628**
	14	4.87	4.63	**0.914**
C13:0 (Tridecanoic)	1	0.00	0.00	-
	10	0.08	0.00	**0.854**
	14	0.10	0.13	**0.936**
C14:0 (Myristic)	1	11.79	11.96	**0.941**
	10	12.04	12.48	**0.917**
	14	12.96	13.02	**0.951**
C15:0 (Pentadecanoic)	1	0.40	0.63	**0.204**
	10	0.71	0.52	**0.221**
	14	0.86	0.84	**0.748**
C16:0 (Palmitic)	1	34.95	33.42	**0.836**
	10	33.23	33.75	**0.904**
	14	33.63	35.59	**0.805**
C17:0 (Heptadecanoic)	1	0.41	0.41	**0.974**
	10	0.36	0.32	**0.903**
	14	0.30	0.33	**0.914**
C18:0 (Stearic)	1	9.11	8.85	**0.794**
	10	11.73	10.72	**0.657**
	14	11.13	10.14	**0.690**
Total SFA[a]	1	69.17[b]	72.27[b]	**0.415**
	10	73.80[ab]	75.20[ab]	**0.514**
	14	78.96[a]	79.46[a]	**0.758**

Note: No significant differences between groups (P > 0.05) were observed regarding samples collection days, as well as for the sum of saturated fatty acids (Total SFA).
[a] In each fatty acid or sum (Total) indicates a significant difference over the time for these variables in each group.

Schauss et al., 2009). Anthocyanin exerts potent antioxidant and anti-inflammatory actions that contribute to decreasing pro-inflammatory actions (Rosso et al., 2008; Menezes et al., 2008). Another important result of our study was the increased concentration of globulins in the açaí group, possibly related to the increase of immunoglobulins that are important to maintain health of the animal and its mammary gland.

In summary, açai oil in the diets of lactating sheep had antioxidant and anti-inflammatory actions. The antioxidant and anti-inflammatory effects can be reflected in the milk quality, because of reduced lipoperoxidation and increased antioxidant capacity, effects that are beneficial to the health of consumers of milk and may even increase the shelf life of the product. Increased milk production can be related to the greater energy production and lower fat mobilization. Therefore, inclusion of 2% açaí oil in the diets of dairy sheep under heat stress can improve animal health, consequently increasing milk performance and improving milk quality, as well as producing product with nutraceutical characteristics. The inclusion of açai oil in sheep diets improved the antioxidant and anti-inflammatory activity in serum and milk, and improved milk production and quality, despite the reduced concentration of fat and total solids in the milk of sheep under heat stress. Açai oil can be used to improve the health and performance of dairy sheep.

Table 4
Unsaturated fatty acids profile in the milk of sheep supplemented with açai oil (AÇAI; *Euterpe oleracea*) or soybean oil (SOY).

Variables	Day	SOY	AÇAI	P-values
C17:1 (cis-10-Heptadecenoic)	1	0.15	0.11	**0.887**
	10	0.00	0.00	-
	14	0.00	0.00	-
C16:1 (Palmitoleic)	1	0.80	0.72	**0.658**
	10	0.75	0.58	**0.224**
	14	0.48	0.59	**0.541**
C18:1n11t (Vacenic)	1	1.15	1.66	**0.657**
	10	1.79	1.40	**0.593**
	14	1.08	1.01	**0.847**
C18:1n9c (Oleic)	1	27.54	25.79	**0.597**
	10	21.68	21.31	**0.901**
	14	18.03	17.41	**0.874**
C20:1n9 (cis-11-Eicosenoic)	1	0.00	0.27	**0.185**
	10	0.29	0.23	**0.850**
	14	0.00	0.04	**0.700**
Total MUFA[a]	1	29.64[a]	28.28[a]	**0.814**
	10	24.22[ab]	23.29[ab]	**0.821**
	14	19.60[b]	19.22[b]	**0.943**
C18:2n6c (Linoleic)	1	1.06	1.08	**0.934**
	10	1.68	1.29	**0.892**
	14	1.36	1.25	**0.825**
C18:3n3 (α-Linolenic)	1	0.00	0.00	-
	10	0.00	0.00	-
	14	0.18	0.20	**0.869**
C20:4n6 (Arachidonic)	1	0.13	0.10	**0.824**
	10	0.00	0.00	-
	14	0.00	0.06	**0.714**
Total PUFA[a]	1	1.19[b]	1.18[b]	**0.935**
	10	1.68[a]	1.29[ab]	**0.245**
	14	1.54[ab]	1.48[a]	**0.785**

Note: No significant differences between groups (P > 0.05) were observed regarding samples collection days, as well as for the sum of monounsaturated (Total MUFA) and polyunsaturated (Total PUFA).
[a] In each fatty acid or sum (Total) indicates a significant difference over the time for these variables in each group.

Table 5
Hemogram of sheep supplemented with açai oil (AÇAI; *Euterpe oleracea*) or soybean oil (SOY).

Variables	Day	SOY	AÇAI	P-values
Erythrocytes[#] (x10^6 µL)	1	5.07 (1.14) [b]	5.29 (1.10) [b]	**0.658**
	10	6.31 (1.32) [ab]	6.61 (1.12) [ab]	**0.854**
	14	7.75 (0.98) [a]	7.64 (0.25) [a]	**0.847**
Hematocrit[#] (%)	1	28.3 (3.6)	27.0 (3.4) [b]	**0.954**
	10	29.7 (5.0)	29.2 (4.9) [ab]	**0.937**
	14	33.2 (2.7)	33.5 (3.9)[a]	**0.944**
Hemoglobin (mg/dL)	1	8.95 (1.21)	8.2 (1.27)	**0.698**
	10	8.82 (1.54)	8.55 (1.72)	**0.853**
	14	10.05 (1.29)	9.7 (1.26)	**0.741**
Leukocytes[#] (x10^3 µL)	1	4.81 (1.18)	4.78 (0.92)[a]	**0.894**
	10	5.20 (1.24)	4.56 (1.34)[ab]	**0.201**
	14	5.72 (1.18)	3.51 (0.65)[b]	**0.036***
Lymphocytes (x10^3 µL)	1	2.20 (0.71)	2.26 (0.42)	**0.914**
	10	2.64 (1.23)	2.23 (1.12)	**0.854**
	14	2.16 (0.57)	1.47 (0.38)	**0.050***
Neutrophils (x10^3 µL)	1	2.49 (0.73)	2.40 (0.63)	**0.798**
	10	2.44 (0.55)	2.58 (0.73)	**0.774**
	14	3.40 (1.31)	1.93 (0.60)	**0.001***
Monocytes (x10^3 µL)	1	0.11 (0.06)	0.12 (0.05)	**0.852**
	10	0.04 (0.05)	0.62 (1.22)	**0.498**
	14	0.34 (0.24)	0.21 (0.12)	**0.245**
Eosinophils (x10^3 µL)	1	0.0 (0.00)	0.0 (0.0)	**0.999**
	10	0.04 (0.05)	0.01 (0.01)	**0.904**
	14	0.02 (0.05)	0.0 (0.0)	**0.927**

*P ≤ 0.05 indicates a significant difference between groups. Note: [#] identifies variables that differed over time, and the difference between determined periods (days 1, 10 and 14) was identified by different letters in the same column ([a, b]).

Table 6
Serum biochemistry of sheep supplemented with açai oil (AÇAI; *Euterpe oleracea*) or soybean oil (SOY).

Variables	Day	SOY	AÇAI	P-values
Glucose[#]	1	65.4 (8.1)	52.7 (13.7) [b]	**0.125**
(mg/dL)	10	65.5 (22.4)	78.87 (12.6)[ab]	**0.456**
	14	62.7 (7.3)	105.6 (24.5)[a]	**0.001***
Cholesterol	1	78.3 (22.0)	80.5 (23.2)	**0.747**
(mg/dL)	10	95.5 (25.0)	97.11 (38.8)	**0.579**
	14	80.5 (22.8)	73.5 (23.0)	**0.652**
Triglycerides[#]	1	29.6 (11.2)	28.4 (5.0)[a]	**0.802**
(mg/dL)	10	29.5 (6.8)	30.0 (7.4)[a]	**0.872**
	14	24.6 (9.8)	11.5 (3.8) [b]	**0.050***
Total protein	1	9.6 (1.4)	9.8 (1.57)	**0.901**
(g/dL)	10	10.3 (2.1)	10.8 (1.8)	**0.853**
	14	8.40 (0.55)	8.78 (0.8)	**0.889**
Albumin	1	3.27 (0.68)	3,20 (0.49)	**0.787**
(g/dL)	10	3.33 (0.67)	3.85 (0.69)	**0.447**
	14	2.95 (0.4)	2.65 (0.36)	**0.841**
Globulin	1	6.32 (1.34)	6.66 (1.25)	**0.852**
(g/dL)	10	7.06 (2.0)	6.96 (2.2)	**0.745**
	14	5.45 (0.30)	6.13 (0.27)	**0.039***
Urea[#]	1	36.3 (7.68)[a]	41.22 (11.9)[a]	**0.745**
(mg/dL)	10	26.81 (7.6) [b]	21.5 (7.1) [b]	**0.198**
	14	39.5 (8.2)[a]	28.5 (5.1) [ab]	**0.045***

*P ≤ 0.05 indicates a significant difference between groups. Note: [#] identifies variables that differed over time, and the difference between determined periods (days 1, 10 and 14) was identified by different letters in the same column ([a, b]).

Ethical note

The project was approved by the animal ethics committee of UDESC, under protocol number 2854200317.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.07.007.

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