ANIMAL SCIENCE

Addition of açai oil during the close-up dry period of Holstein cows improves colostrum quality and immune responses of their calves

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Abstract: This study evaluated of the effects of açai oil during the close-up dry period of Holstein cows on colostrum quality, as well as on the immune and antioxidant responses of their calves. Sixteen multiparous cows were assigned randomly to two treatments: 1) CONTROL (n = 8) - 4.48% of soybean oil/concentrate; 2) AÇAI (n =8) - 4.48% of açai oil/concentrate. Cows fed with açai oil had greater ($P \leq 0.04$) colostrum concentrations of immunoglobulins (Ig) G (1st and 2nd milking), IgG heavy chains, IgA (only at 1st milking), alpha-lactalbumin (1st milking), total protein, and antioxidant capacity against peroxyl radicals (only at 1st milking). Cows fed with açai oil had greater serum concentrations of globulin (only on the day of calving) and total protein (only on the day of calving) ($P = 0.03$). Calves born of cows fed with açai oil had greater serum concentrations of total protein (only 24 and 48 h after calving) and serum concentration of IgG heavy chain (only 24 h after calving) and globulin (only 24 and 48 h after calving) ($P = 0.01$). These data suggest that the addition of açai oil in the cow feed during the close-up dry period boosted immunity in their calves by altering the composition of colostrum.

Key words: açai oil, antioxidant system, colostrum, cows, immunity, prepartum.

INTRODUCTION

During pregnancy, the transfer of immunoglobulins (Ig) from the cow to the fetus is minimal or absent, however, Ig can be transferred from the cow to the calf after calving through colostrum (Boulton et al. 2015). Immunoglobulins from colostrum are essential for the calf to obtain their first antibodies. Several factors could affect the concentration of Ig in the colostrum, including breed, age of the dam, season of the calving, prepartum vaccination, dry period length, volume of colostrum produced in the first milking, delayed colostrum collection and the prepartum diet (Godden et al. 2019). As a result, the animal feed industry and researchers have been looking for food alternatives to bolster the health of cows, produce colostrum with larger amounts of antibodies, and thereby carry out efficient immunization of newborns via colostrum. It is well known that the addition of antioxidants and immunostimulants such as selenium and vitamin E in the diet of prepartum dairy cows increased Ig concentrations in colostrum (Pavlata et al. 2004). Moreover, studies have reported that plant materials enhance the activity of cells of the innate immune system and modify host responses (Holderness et al. 2007, Graff et al. 2009).

Researchers describes a potent immunomodulatory activity from açai

(*Euterpe oleracea*) on monocyte and γδ T cell populations, as well as açai polysaccharide induced myeloid cell recruitment and IL-12 production (Holderness et al. 2011). According to literature, the açai fruit is a popular nutritional supplement that purportedly enhances immune system function (Holderness et al. 2011, Khoo et al. 2017). From this fruit, by-products have been produced, characterized and commercialized, as highlighted here for the açai oil. This is an excellent source of anthocyanins, which are potent antioxidants belonging to the family of flavonoids responsible for the coloring of the fruit (Cedrim et al. 2018). Study verified that the polysaccharide fraction of açai induced robust immune cell stimulatory activity in human, mouse, and bovine peripheral blood mononuclear cells cultures (Holderness et al. 2011).

A recent study by our research group showed that the açai oil supplementation in heat-stressed sheep increased milk production and improved immune and antioxidant responses (Santos et al. 2019). However, we are unaware of studies evaluating the effects of the addition of açai oil in the prepartum cow diets on subsequent colostrum quality and immune response of theirs calves. Our hypothesis was that açai oil in feed would improve the colostrum quality and boost the immune response of calves consuming this colostrum; because in sheep that consumed açai oil the concentration of total antioxidants increased considerably in milk (Santos et al. 2019). Therefore, the objective of this study was to determine the effects of the addition of açai oil during the close-up dry period of Holstein cows on colostrum quality, as well as on the immune and antioxidant responses of their calves.

MATERIALS AND METHODS

Oils

Açai oil was extracted by cold pressing, according to manufacturer's information (Gran oils, São Paulo, SP, Brazil) and soybean oil was purchased at a local supermarket (Soya, Brasília, DF, Brazil).

The fatty acid concentrations of açai and soybean oil were analyzed using approximately 30 mg of oil for derivatization in fatty acid methyl esters (FAME) according to Hartman & Lago (1973) with some modifications described by Santos et al. (2019). The FAME were analyzed using gas chromatography with a flame ionization detector (GC-FID; model Star 3600, Varian, USA) by injecting 1 μL of oil into a split/splitless injector with a ratio of 20:1, heated at 250 °C. Identification of FAME was performed by comparing sample retention times with those of FAME Mix-37 (P/N 47885-U; Sigma-Aldrich, USA). The results were expressed as percentage of the total area with consideration of FID correction factors.

The fatty acid profile of soybean and açai oil consisted of palmitic acid (C16:0) [soybean oil 14.3%; açai oil 11.0%], stearic acid (C18:0) [soybean oil 3.23%; açai oil 1.86%], oleic acid (C18:1n9c) [soybean oil 30.8%; açai oil 38.7%], linoleic acid (C18:2n6c) [soybean oil 44.3%; açai oil 44.9%], and linolenic acid (C18:3n3) [soybean oil 5.06%; açai oil 3.71%].

Location and animals

This experiment was conducted during winter at a commercial dairy farm in Tunápolis, Santa Catarina, Brazil (Latitude: 26° 58' 28'' South; Longitude: 53° 38' 20'' West). The farm has a vaccination schedule for infectious bovine rhinotracheitis, bovine viral diarrhea, and bovine leptospirosis (commercial vaccine: Poliguard®, Vallée, Brazil.) At 6-month intervals; and a commercial vaccine to clostridiosis at

12-month intervals (Ourovac®, Ouro Fino, Brazil); vaccines that were applied in the sixth month of cows' gestation. Gastrointestinal parasite control on the farm is performed once a year and approximately 30 days prior to calving using doramectin. Tick control was accomplished by spraying when needed using commercial product based on cypermethrin (moderate or high infestations). It is important to make it clear that all these procedures (vaccine and antiparasitic) occurred at 18 cows used in this study, which allows to guarantee that the changes described in the "results" section were due to the consumption of a diet containing açai oil.

In the prepartum period, cows were housed in a covered freestall barn (200 m^2) with *ad libitum* access to water. For the individual feeding of each cow, it was contained in its feeder with the help of kennel.

During the experimental period, the minimum temperature ranged between - 0.1 ºC and 11.2 ºC. These temperatures were within the thermoneutral zone for cows (0 ºC and 16 ºC), but not for calves (12 ºC to 25 ºC) (NRC 2001).

Experimental design

Sixteen Holstein multiparous cows (third (n = 10) or fourth gestation (n = 6)) were used during the prepartum period. Based on previous lactations, peak milk production ranged from 30 to 35 L/day. Cows were assigned randomly to one of two treatments (eight cows/treatment): 1) concentrate with 4.48% soybean oil (CONTROL group) or 2) concentrate with 4.48% açai oil (AÇAI group) for 20.9 ± 2.6 days prior to expected calving. Oils used in this study had similar energy values (108 kcal), according to the manufacturer's guaranteed analysis. The amount of oil (soy and açai) in the diet was based on a pilot study (unpublished data).

The basal diet provided before the experimental period was exactly the same that was used during the study (Table I), but without oil supplementation. During pre-partum experimental period, the cows were fed twice a day (06:00 and 17:00 h), and the amount of food being divided equally as describe in Table I. First, half of the concentrate per day containing oil (soy or açai) was supplied to the cows; with 100% of this food ingested by all animals within 15 min. Then, silage and hay (*Cynodon* spp.) mixed in the feeder were supplied. We waited for approximately 30 min of ingestion of this feed, then the cow was released to drink water from the pen, and then immediately returned to her feeder to continue eating for another 60 min. Subsequently, the cows are free in the collective stall until the next feeding.

Samples of the total feed provided to cows (concentrate, silage and hay) from both groups were collected and analyzed according to AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ether extract (EE), method 920.39; and ash, method 942.05. The concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured according to the methodology of Van Soest et al. (1991) without the addition of sodium sulfite or alpha-amylase. The concentrations of total digestible nutrients (TDN) were calculated according to Weiss et al. (1991) (Table I).

Management of calves after birth

Dietary treatments were given for 20.9 ± 2.6 days prior to calving, and calf immune response was evaluated for 5 days after birth. Calves were removed from their mothers immediately after calving such that no suckling occurred, then were weighed on a digital scale. Then, calves were housed in individual pens and received feed Subsequently, navel prophylaxis was performed with a 5% iodized alcohol solution.

Table I. Ingredients and chemical composition of the diet offered to dairy cows receiving either soybean (Control) or açai (Treated) oils during the close-up dry period (20.9 ± 2.6 days prior to calving).

Feeds (kg)	As fed (kg/cow/day)	Dry matter (DM; kg/cow/day)
Corn silage	6.40	2.39
Hay	2.70	2.29
Concentrate[1]	2.70	2.42

Ingredients (g/kg)	Concentrates	
	CONTROL	AÇAI
Ground corn	500.80	500.80
Soybean meal	346.00	346.00
Premix[1]	108.40	108.40
Soybean oil	44.80	0.00
Açai oil	0.00	44.80

Chemical composition[2]	Corn silage	Hay	Concentrates[1]	
			CONTROL	AÇAI
Dry matter (DM), g/kg	371.20	848.70	897.52	894.61
g/kg of DM				
Crude protein	81.62	60.65	203.83	201.83
NDF	346.13	707.60	80.10	80.21
ADF	211.51	332.32	13.41	13.63
EE	35.07	11.99	60.97	59.52
Ash	68.71	79.20	134.60	118.61
TDN[3]	714.72	605.2	782.62	796.23

[1]The premix had: Biotin (min. 80 mg), calcium (min./max 55/100 g), chlorine (min. 313.2 g), cobalt (min. 5.6 mg), copper (min. 556 mg), chromium (min. 32 mg), sulfur (min. 90 mg), iron (min. 223.5 mg), fluorine (max. 150 mg), phosphorus (min. 20 mg), iodine (min. 14.2 mg), magnesium (min. 20 g), manganese (min. 692 mg), Monensin Sodium (min. 804 mg), Mineral material (max. 800 g), selenium (min. 15 mg), organic selenium (min. 7.51 g), sodium (min. 32 g) , Vitamin A (min. 300003 IU), Vitamin D3 (min. 81601 IU), Vitamin E (min. 8280 IU), and zinc (min. 921 mg).
[3]NDF (neutral detergent fiber), ADF (acid detergent fiber), EE (ether extract) and TDN (total digestible nutrients).
[2]Calculated as described by Weiss et al. (1991).

Postpartum milking: colostrum/milk

The first milking of the cows was performed using a mechanical milking machine between 1 to 2 h after calving. The interval between the first and the second milking was approximately 8 h. The quantification of colostrum was performed (first and second milking), using a mechanical milking machine connected to a pot.

Milk production of cows was measured only 4 days postpartum after mechanical milking and reported as total volume (L) of milk.

Intake of colostrum and feed

Calves were housed in individual pens and received colostrum/milk. Newborn calves received colostrum from their mothers within 2 hours of birth. Colostrum was provided at 5% of body weight (BW) using a bottle. Subsequently, transition milk (2nd milking) was used to feed the calves up to 8 hours after the first feeding at 10% of BW. After birth, calves were fed with 4 L/d (divided in two feedings/day) of transition milk (between at days 2 and 4) and milk (between

at days 4 and 5) obtained from the total milk at the end each milking. Each calves consumed its mother's milk during the top trial period. The intake of water and concentrate (from the third day of age) *ad libitum*.

It is important to note that each calf was accompanied for only 5 days after birth, and after that period the animals remained on the farm under the responsibility of the producer.

Sample collection

Blood

Cow blood samples were collected from the coccygeal vein into 10-ml blood collection tubes without sodium heparin 21 days before expected calving (before the addition of oil supplements) and on the day of calving. Calf blood samples were collected from the jugular vein into 10-ml blood collection tubes without anticoagulant at 1 h (immediately after calving), 24 h, and 120 h after calving. Tubes (cows and calves) were centrifuged at 5100 × g for 10 min for serum separation. Serum were stored in microtubes (1.5 ml each) at –20 °C for further analysis.

Colostrum/milk

Samples of first and second milking (occurred at 8 h intervals mean), samples two of 250 and 2 ml of colostrum were collected and stored in tubes. Samples were stored in microtubes (2 ml each) at –20 °C for further analysis. Samples of 250 ml were used to quantify immunoglobulin concentration using a colostrometer.

Field analysis

In our study, we chose to use measurements commonly used on farms, both to assess colostrum quality and calf immunization. These methodologies are widely known and used, however, they have less sensitivity. Nevertheless,

we believe it is important to measure technical variables.

Colostrometer

Colostrum Ig concentrations were evaluated between 20 and 25 ºC, using a colostrometer (Suprivet, Divinópolis, MG, Brazil). Concentrations were determined by the correlation between the specific gravity of the colostrum and the concentration of immunoglobulin, according to the scale proposed by Fleenor & Stott (1980).

Refractometer

Serum protein concentrations in calves were measured after blood collection and centrifugation using a refractometer (Suprivet, Divinópolis, MG, Brazil) as described by Caldas et al. (2015).

Laboratory analysis

Proteinogram

Serum

Protein fractionation was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis according to a technique described by Fagliari et al. (1998) using mini-gels (10 x 10 cm). The gels were stained with Coomassie blue and photographed to identify and quantify protein fractions using Labimage1D software (Loccus Biotechnology). Standards containing protein fractions with molecular weights between 10 and 250 kDa (Kaleidoscope - BIORAD) were used as references for the identification of protein fractions. For quantification, the total protein content previously obtained using the biuret technique was used as a reference.

Colostrum

To perform the proteinogram, milk serum was obtained using the technique described by

Schalm et al. (1971). Initially, colostrum samples after thawing in a 37 °C water bath were homogenized by vortexing; for every 1,000 µL of milk, 75 µL of 10% renin solution were added, kept in a water bath at 37 °C for about 20 minutes and centrifuged at 21,000 x g for ten minutes. The intermediate fraction resulting from the three-phase solution, corresponding to the whey, was fractionated in Eppendorf tubes and subjected to analysis. The determination of total whey protein was performed using the Biuret methodology.

The separation of milk proteins was performed using polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE), as described by Laemmli (1970) and Fagliari et al. (1998). After the end of the running time, the gel was stained with Coomassie Blue until the bands were marked, with the excess of the dye removed using 7% acetic acid. The gels were subsequently photographed and the identification and quantification of protein fractions was performed using Labimage1D (Loccus Biotecnologia) software. The reference protein ladder contained fractions with molecular weights between 10 and 250 KD (Kaleidoscope - BIORAD). Depending on the protocol used, immunoglobulin G (IgG) was separated into two chains, light chain (light IgG) and heavy chain (heavy IgG), resulting from the use of 2-mercaptoethanol in the buffer solution, used in the preparation of the samples for SDS-PAGE. For quantification, the total protein content previously obtained using the Biuret technique.

Serum biochemistry

Serum total protein and albumin were measured using a semi-automated analyzer (BioPlus 2000®) with commercial kits (Analisa®, Gold Analisa Diagnóstica, Belo Horizonte, Brazil). Globulin concentrations were obtained using the following formula: total protein – albumin.

Oxidants and antioxidants

Lipoperoxidation (LPO)

Lipid peroxidation (LPO) analysis (FOX-based) was performed as described by Monserrat et al. (2003) with modifications to colostrum and serum. This method is based in the reaction of hydroperoxides present in the colostrum sample (100 µL) with Fe^{2+} ($FeSO_4$–0.25 mM) in an acidic medium (H_2SO_4–0.025 mM) in the presence of the dye Xylenol Orange (100 µM–Sigma Aldrich). This samples were diluted (1:9 w/v) in cold methanol (100%) and then centrifuged at 1000 × g for 10 min at 4 °C. LPO was measured in the supernatants using a microplate reader at 550 nm. Cumene hydroperoxide (CHP–3 µM–Sigma Aldrich) was used as standard. Results were expressed in nmol of CHP/mL.

Reactive oxygen species (ROS)

ROS concentrations in serum were determined as described by LeBel et al. (1992) with modifications describes by Tarouco et al. (2017). Sample aliquots (10 µL) were incubated in a microplate in a medium containing cold buffer (127.5 µL–HEPES 30 mM, KCl 200 mM and $MgCl_2$ 1 mM). After that, 2',7'-dichlorodihydrofluorescein diacetate (10 µL–H2DCF-DA, 16 µM, Molecular Probes) was added. The reactive oxygen molecules present in the sample react with 2',7'-dichlorodihydrofluorescein diacetate generating a fluorochrome that is detected fluorimetrically (SpectraMax i3x - Molecular Devices) employing wavelengths of 485 nm (excitation) and 520 nm (emission). The fluorescence areas were calculated according to a quadratic equation and these values were used as an indication of the concentration of ROS in each sample. The results were expressed as U DCF/mg of protein.

Antioxidant capacity against peroxyl radicals (ACAP)

The ACAP analyses followed the protocol described by Amado et al. (2009) without modifications to colostrum. This methodology is based on the thermal decomposition (37 °C) of ABAP (2,2'-azobis(2-methylpropio-namidine) dihydrochloride; 20 mM; Sigma-Aldrich), which generates peroxyl radicals. These radicals oxidize $H_2DCF-DA$ to generate afluorochrome that is detected fluorimetrically, employing wavelengths of 485 nm (excitation) and 520 nm (emission). These generated peroxyl radicals are intercepted by the antioxidants present in the biological sample, reducing the generation of fluorescence. Briefly, sample aliquots (10 µL) were incubated in a microplate in a medium containing cold buffer (127.5 µL, HEPES 30 mM, KCl 200 mM and $MgCl_2$ 1 mM). After that, $H_2DCF-DA$ (10 µL, 16 µM) was added in each well. Samples aliquots were incubated in the presence or in the absence of ABAP (7.5 µL–4 mM). Detection of fluorescence (with and without ABAP) was performed during 40 min at 37 °C and results were expressed by the relative fluorescence area (fluorescence × time) and ACAP was calculated according to the equation below: ACAP = 1/ [(fluorescence area with ABAP–area without ABAP)/area without ABAP]. The results was expressed as U.F./mg of protein.

Superoxide dismutase (SOD)

SOD activity was determined according to Marklund & Marklund (1974) by estimating the percent inhibition of pyrogallol auto-oxidation by the enzyme at 420 nm. For this, 2.74 mL of Tris-HCl buffer (50 mM, pH 8.2) containing 1 mM each of DETAPAC and EDTA was added to a test tube, to which 0.2 mL of enzyme extract was added. The reaction was initiated by adding 60 µL of pyrogallol (0.2 mM of pyrogallol dissolved in 10 mM HCl) and the change in absorbance was recorded at 420 nm after 6 min of incubation at room temperature. The activity of enzyme was expressed as expressed as SOD units/ mg of protein.

Glutathione S-transferase (GST)

The activity of GST in serum was measured according to Mannervik & Guthenberg (1981) with small modifications. GST activity was measured by the rate of formation of dinitrophenyl-S-glutathione at 340 nm in a medium containing 50 mM potassium phosphate, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. An aliquot of 10 µL of sample was added in a well in a microplate (96-well) with 230 µL of phosphate buffer (100 mM) containing CDNB (0.77 mM–Sigma Aldrich) and GSH (1.0 mM–Sigma Aldrich). The results are expressed in units of GST, which represents the amount of enzyme required to conjugate 1 µmol of CDNB per mg of protein at 25 °C and pH 7.0 The results were calculated and expressed as U GST/mg of protein.

Glutathione peroxidase (GPx)

The glutathione peroxidase (GPx) activity method was described by Wendel (1981) using tert-butyl-hydroperoxide as substrate. The enzymatic activity was determined through the monitoring of the disappearance of nicotinamide adenosine dinucleotide phosphate (NADPH) at 340 nm. The test was performed by mixing 100 mM potassium phosphate (K_3PO_4) buffer/1 mM EDTA (pH 7.7), 2 mM glutathione, 0.15 U/mL glutathione reductase, 0.4 mM azide, 0.5 mM tert-butyl hydroperoxide, 0.1 mM NADPH and 10 µL of tissue supernatant. One unit of GPx consumes 1 µmol of NADPH per minute. The specific activity was reported as U GPx/ mg of protein.

Ethics Committee

All procedures for this project were approved by the *Ethics Committee for the Use of Animals 443 in Search (CEUA)* of the *Universidade do Estado de Santa Catarina*, under protocol number 6834240518.

Statistical analysis

All dependent variables were tested for normality using the Univariate procedure in SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and variables that were not normally distributed were log-transformed (serum levels of ACAP and LPO from cows and calves and serum concentration of ROS from calves). All data were then analyzed using the MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Colostrum production in the first day after calving and days receiving the concentrate prior to calving 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, time (quantity of milkings, days of serum collection of cows before calving or hours of serum collection of calves after birth) and treatment × time, using animal (treatment) as random variables and animal (treatment) as subject. All results obtained 21 d prior to calving for each variable were included as covariates in each respective analysis; however, they were removed from the model when $P > 0.10$. The compound symmetric covariance structure was selected for colostrum production and cow serum concentration of albumin and the first order autoregressive covariance structure was selected for all others variables. The covariance structures were selected according to the lowest Akaike information criterion. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. A simple

Pearson correlation using CORR procedure of SAS was performed to determine the interrelation between colostrum concentration of IgG obtained by colostrometer (1st and 2nd milking) × colostrum proteinogram (1st and 2nd milking) and also the serum concentration of total protein obtained by refractometer (0, 24 and 120 h after calving) × serum proteinogram (0, 24 and 120 h after calving) of calves. A simple Pearson correlation was calculated between the colostrum concentration of proteins (only data of the 1st milking) × serum concentration of proteins of calves (only data of 24 h after calving). Significance was defined when $P \leq 0.05$, and tendency toward significance when $P > 0.05$ and ≤ 0.10.

RESULTS

Time in treatment

No differences were detected ($P = 0.85$) between treatments for days receiving concentrate prior to calving (20.75 vs. 21.00 ± 0.93 days, for cows of CONTROL and AÇAI groups, respectively).

Calf body weight

Calf body weight at birth did not differ ($P = 0.83$) between treatments (39.2 vs. 40.6 ± 4.33 kg for calves born of cows of CONTROL and AÇAI groups, respectively).

Colostrum production and quality

No differences were detected ($P \geq 0.55$) between treatments for colostrum production (Supplementary Material - Table SI) and serum concentration of IgG light chain, transferrin, or beta-lactalbumin (Table II). However, cows fed with açai oil had greater ($P \leq 0.04$) colostrum concentrations of IgG (1st and 2nd milking - obtained using the colostrometer; Table SII), IgG heavy chain, IgA (only at 1st milking), alpha-lactalbumin (only at 1st milking), total protein, and ACAP (only at 1st milking), compared to

Table II. Colostrum production and quality of cows fed with soybean (Control) or açai (AÇAI) oil during close-up dry period (20.9 ± 2.6 days prior to calving).

Items[1]	Treatments[2]		P - values		
	CONTROL	AÇAI	Treat	Time	Treat × Time
Colostrum production (L/milking)			0.91	0.01	0.87
1st milking	8.50 (0.60)[A]	8.38 (0.59)[A]			
2nd milking	5.67 (0.60)[B]	5.59 (0.59)[B]			
Average	7.09 (0.59)	6.99 (0.59)			
Colostrum production (L/day; 1st day)	14.17 (1.18)	13.97 (1.18)	0.81	-	-
IgG heavy chain (g/dL)			0.01	0.01	0.45
1st milking	1.26 (0.05)[A]	1.77 (0.06)[A]			
2nd milking	0.51 (0.06)[B]	0.93 (0.06)[B]			
Average	0.89 (0.04)[y]	1.35 (0.04)[x]			
IgG light chain (g/dL)			0.98	0.01	0.80
1st milking	0.60 (0.05)[A]	0.61 (0.06)[A]			
2nd milking	0.30 (0.06)[B]	0.29 (0.06)[B]			
Average	0.45 (0.04)	0.45 (0.04)			
IgA (g/dL)			0.07	0.01	0.04
1st milking	0.75 (0.07)[Ay]	1.05 (0.07)[Ax]			
2nd milking	0.62 (0.07)[A]	0.65 (0.07)[B]			
Average	0.68 (0.06)[y]	0.85 (0.06)[x]			
Transferrin (g/dL)			0.90	0.01	0.50
1st milking	1.09 (0.09)[A]	1.06 (0.10)[A]			
2nd milking	0.63 (0.10)[B]	0.70 (0.10)[B]			
Average	0.86 (0.09)	0.88 (0.08)			
Beta-lactalbumin (g/dL)			0.96	0.01	0.55
1st milking	0.52 (0.04)[A]	0.54 (0.04)[A]			
2nd milking	0.33 (0.04)[B]	0.31 (0.05)[B]			
Average	0.43 (0.03)	0.43 (0.03)			
Alpha- lactalbumin (g/dL)			0.30	0.01	0.01
1st milking	0.32 (0.03)[Ay]	0.47 (0.03)[Ax]			
2nd milking	0.32 (0.03)[A]	0.24 (0.03)[B]			
Average	0.32 (0.02)	0.35 (0.02)			
Total protein (g/dL)			0.01	0.01	0.33
1st milking	7.97 (0.27)[A]	10.76 (0.29)[A]			
2nd milking	3.35 (0.32)[B]	5.55 (0.32)[B]			
Average	5.66 (0.21)[y]	8.15 (0.22)[x]			
ACAP (U.F/mg of protein)			0.01	0.01	0.01
1st milking	0.96 (0.16)[Ay]	2.39 (0.17)[Ax]			
2nd milking	1.04 (0.17)[A]	1.04 (0.17)[B]			
Average	1.00 (0.12)[y]	1.72 (0.12)[x]			
LPO (nmol/mL)			0.05	0.71	0.28
1st milking	3894.44 (456.93)	3328.61 (488.48)			
2nd milking	4238.69 (488.02)	2644.10 (488.48)			
Average	4066.57 (352.74)[x]	2986.35 (365.79)[y]			

[1]Colostrum from the first (1st milking) and second (2nd milking) milking after calving, immunoglobulin G (IgG) and A (IgA), antioxidant capacity against peroxyl radicals (ACAP) and lipid peroxidation (LPO).

[2]The treatments CONTROL or AÇAI represents the inclusion of 4.48% soybean or acai oil, respectively, for 20.9 ± 2.6 days prior to calving.

[x-y]Within a row, without a common superscript differ ($P ≤ 0.05$) or tends to differ ($P ≤ 0.10$).

[A-B]Within treatment, without a common superscript differ ($P ≤ 0.05$) or tends to differ ($P ≤ 0.10$).

control cows (Table II). Cows fed with açai oil had lower serum levels of LPO than control cows (P = 0.05) (Table II).

Cow serum protein concentrations

No differences were detected ($P \geq 0.11$) between treatments for serum concentration of albumin (Table III). However, cows fed with açai oil had greater serum concentration of globulin (only on the day of calving) and total protein (only at day of calving), compared to control cows (P = 0.03) (Table III).

Calf serum protein concentrations

Calves born of cows fed with açai oil had greater serum concentration of total protein (only 24 and 48 h after calving; Table I) and serum concentration of IgG heavy chain (only 24 h after calving) and globulin (only 24 and 48 h after calving), compared to calves born of control cows (P = 0.01) (Table III). Calves born of cows fed with açai oil tended to have greater serum concentrations of total protein, compared to calves born of control cows (P = 0.10) (Table III). No differences between treatments were detected for serum concentration of IgG light chain and albumin ($P \geq 0.12$) (Table III).

Correlations between proteins from colostrum and serum of calves

Significant positive Pearson correlations were calculated between the colostrum concentrations of IgG obtained using the colostrometer and a series of colostrum protein variables obtained by proteinogram ($P \leq 0.05$; Table II). Significant positive Pearson correlations were detected between serum concentrations of total protein of calves obtained using the refractometer and a series of serum proteins variables obtained by proteinogram (P = 0.01; Table II). A series of proteins in the colostrum had, or tended to have ($P \leq 0.07$) positive Pearson correlations with a series of proteins in the serum of the calves (Table IV). Pearson correlation coefficients (PCC) among colostrum IgG × plasma/serum concentrations of proteins were showed in Table SII, as well as PCC among plasma total protein levels × other protein in serum, and Colostrum × plasma/serum of offspring.

Oxidant and antioxidant status of calves

Calves born of cows fed with açai oil had greater (P = 0.01) serum concentration of GST and tended to have greater (P = 0.01) serum concentration of GPx, compared to calves born of control cows (Table V). No differences between treatments were detected ($P \geq 0.11$) for serum levels of SOD, ROS, ACAP, or LPO (Table V).

DISCUSSION

Addition of açai oil in the feed of prepartum cows increased the concentrations of serum total protein on the day of calving and the concentration of Ig in colostrum. Consequently, calves born of cows fed with açai oil had higher levels of globulins, IgG heavy chain, IgA and greater activity of antioxidant enzymes, GPx and GST. A healthy mammary gland is important for maximal colostrum/milk yield and quality (Martí et al. 2013) as well as for the immunity of the calf. Poor quality colostrum can increase the susceptibility of a calf to disease, increasing mortality and consequently lowering profitability (Ribeiro et al. 1983, Braun & Tennant 1983). Consistent with other beneficial precalving management strategies, açai oil positively impacted colostrum quality by increasing Ig (Andrew & Otterby 2001). Prepartum diets that provide good sources of energy, protein and vitamins can improve fetal development and augment colostrum yield and quality (Puppel et al. 2019). Similarly, diets for prepartum dairy cows that are enriched with saturated

Table III. Serum concentration of proteins of cows fed with soybean (Control) or açai (AÇAI) oil during close-up dry period (20.9 ± 2.6 days prior to calving) and their calves.

Items[1]	Treatments[2]		P – values		
	CONTROL	AÇAI	Treat	Time	Treat × Time
Cows					
Albumin (mg/dL)			0.11	0.01	0.24
21 d prior to calving	2.47 (0.12)^A	2.30 (0.11)^A			
Day of calving	2.90 (0.12)^B	2.50 (0.11)^A			
Average	2.68 (0.11)	2.42 (0.10)			
Globulin (mg/dL)			0.10	0.01	0.01
21 d prior to calving	6.23 (0.22)^A	6.00 (0.22)^A			
Day of calving	5.03 (0.20)^{By}	6.02 (0.20)^{Ax}			
Average	5.63 (0.15)^y	6.01 (0.15)^x			
Total protein (mg/dL)			0.46	0.24	0.03
21 d prior to calving	8.72 (0.20)	8.30 (0.20)			
Day of calving	7.90 (0.19)^y	8.57 (0.19)^x			
Average	8.31 (0.12)	8.44 (0.12)			
Calves					
IgG heavy chain (g/dL)			0.11	0.01	0.01
0 h	0.26 (0.20)^A	0.27 (0.16)^B			
24 h	2.04 (0.16)^{Ay}	3.04 (0.16)^{Ax}			
120 h	1.15 (0.17)^B	0.82 (0.15)^B			
Average	1.15 (0.09)	1.38 (0.08)			
IgG light chain (g/dL)			0.12	0.01	0.15
0 h	0.42 (0.15)^C	0.27 (0.13)^B			
24 h	2.12 (0.14)^A	1.58 (0.14)^A			
120 h	1.13 (0.14)^B	0.62 (0.13)^B			
Average	1.22 (0.11)	0.83 (0.11)			
Albumin (mg/dL)			0.19	0.03	0.30
0 h	2.48 (0.17)^A	2.94 (0.16)^A			
24 h	2.32 (0.17)^A	2.25 (0.17)^B			
120 h	2.62 (0.17)^A	2.77 (0.17)^A			
Average	2.47 (0.09)	2.65 (0.09)			
Globulin (mg/dL)			0.02	0.01	0.01
0 h	2.78 (0.25)^B	2.38 (0.24)^B			
24 h	4.05 (0.25)^{Ay}	5.33 (0.25)^{Ax}			
120 h	4.46 (0.25)^{Ay}	5.08 (0.25)^{Ax}			
Average	3.77 (0.14)	4.26 (0.14)			
Total protein (mg/dL)			0.01	0.01	0.10
0 h	5.26 (0.29)^C	5.33 (0.27)^B			
24 h	6.15 (0.29)^{By}	7.53 (0.29)^{Ax}			
120 h	7.10 (0.29)^{Ay}	7.83 (0.29)^{Ax}			
Average	6.17 (0.17)^y	6.90 (0.17)^x			

[1]Colostrum from the first (1st milking) and second (2nd milking) milking after calving, immunoglobulin G (IgG) and A (IgA), antioxidant capacity against peroxyl radicals (ACAP) and lipid peroxidation (LPO).
[2]The treatments CONTROL or AÇAI represents the inclusion of 4.48% soybean or acai oil, respectively, for 20.9 ± 2.6 days prior to calving.
$^{x-y}$Within a row, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).
$^{A-C}$Within treatment, without a common superscript differ ($P \leq 0.05$) or tends to differ ($P \leq 0.10$).

and unsaturated fatty acids have increased Ig concentrations in colostrum (Garcia et al. 2014). One practical implication of our study was that the refractometer and colostrometer appear to be efficient devices for use on dairy farms as evidenced by the positive correlation between colostrum protein levels with calf whey protein concentrations.

The transfer of passive immunity through colostrum is essential for the protection of calves against disease during the first days of life (Barrington & Parish 2001, Chase et al. 2008). The passive transfer of Ig via colostrum occurs because of the permeability of Ig in the intestinal mucosa of ruminants during the first 18 hours of age (Gomes et al. 2017); the greater the concentration of Ig in the colostrum, the greater immune capacity transmitted to the calf. This explains why the increase in colostrum concentrations of globulins and Ig increased serum total proteins in calves born to cows fed

with açai oil. For the calf to reach adequate levels of immunity, it is necessary for the calf to ingest a sufficient quantity and quality of colostrum at the right time (Bolzan et al. 2010, Feitosa et al. 2010). Consistent with results of the present study, serum IgG concentrations in calves 24 to 30 h after consumption of colostrum were higher in calves born to cows supplemented with fatty acids (unsaturated and saturated) than calves born to cows provided the control diet (Garcia et al. 2014).

There are various classes of Ig, all of which are formed by heavy chains (IgG, IgA, IgM, IgD and IgE) and light chains (kappa or lambda) (Silva et al. 2008). In the present study, cows consuming feed containing açai oil had greater concentrations of IgG heavy chain, IgA and α-lactalbumin in colostrum. The transfer of passive immunity also depends on the calf's intestinal absorption capacity (Bolzan et al. 2010); greater concentrations of IgA in colostrum

Table IV. Pearson correlation coefficients among colostrum × serum concentrations of proteins of calves.

Variables[1]	Pearson correlation coefficients	*P* - values
Colostrum × serum of calves		
Colostrum IgG heavy chain (g/dL)		
× Serum IgG heavy chain (g/dL)	0.68	0.01
× Serum globulin (mg/dL)	0.58	0.06
× Serum total protein (mg/dL)	0.67	0.02
Colostrum IgA (g/dL)		
× Serum globulin (mg/dL)	0.60	0.06
× Serum total protein (mg/dL)	0.58	0.06
Colostrum alpha-lactalbumin (g/dL)		
× Serum IgG heavy chain (g/dL)	0.59	0.03
× Serum globulin (mg/dL)	0.83	0.01
× Serum total protein (mg/dL)	0.67	0.02
Colostrum total protein (g/dL)		
× Serum IgG heavy chain (g/dL)	0.55	0.05
× Serum globulin (mg/dL)	0.75	0.01
× Serum total protein (mg/dL)	0.70	0.02

[1]**Immunoglobulins G (IgG) and A (IgA). Only show the variables that differ (*P* ≤ 0.05) or tended to differ (0.05 > *P* ≤ 0.10).**

can increase the protection and intestinal absorption of the calf, because IgA protects the intestinal mucosa from entry of possible pathogens while the calf is ingesting colostrum (Tizard 2008).

Nutritional requirements increase during the prepartum period, in turn increasing cellular respiration; this may lead to ROS production in excess of levels matching endogenous antioxidant capacity, thereby leading to oxidative stress (Weiss 1998). Oxidative stress during the transition period increases susceptibility to health problems such as immunodeficiency, leading to decreased colostrum and milk yields (Goff 2006, Sordillo & Aitken 2009). In the current study, açai oil increased the ACAP of colostrum, resulting in a reduction in lipoperoxidation in the transition milk. The greater ACAP in colostrum occurred because açai oil has high concentrations of antioxidants with phytochemical compounds, including flavonoids, anthocyanins, and pro-anthocyanins (Brito et al. 2007, Novello et al. 2015), all of which are important components for defense against oxidative stress (Schauss et al. 2006). Similarly, antioxidants (selenium and vitamin E) in prepartum dairy cows increased the concentration of immunoglobulins in colostrum (Pavlata et al. 2004), contributing to improvements in calf immunity, as was observed in our study. The reduction of oxidative stress and the increase of antioxidants in the colostrum of cows receiving açaí oil explains the increase in the concentration of α-lactalbumin. According to Boehmer et al. (2010), α-lactalbumin reduces when an inflammatory process occurs. The increase in α-lactalbumin concentration in the present study is a beneficial effect because this participates in lactose biosynthesis, milk production, and secretion (Farrell Jr et al. 2004).

Açai increased the activity of GPx and GST. Supplying a açai pulp supplement to rats increased concentrations of the antioxidant enzyme, glutathione S-transferase (Souza et al. 2010). Providing a diet containing 2% açai oil to diabetic rats decreased lipid peroxidation, subsequently reducing oxidative stress (Guerra et al. 2011). According to same authors, the increase in total glutathione may be related to the gene expression of enzymes involved in the antioxidant defense system, suggesting a role in cellular redox signaling.

In the present study, cows were not under cold stress; however, the calves were (NRC 2001). Cows within the zone of thermoneutrality can maintain production; however, outside the thermoneutral zone, the animal must use energy to dissipate or gain heat, which significantly reduces energy for other essential metabolic activities. Energy expenditure due to maintenance of body temperature can negatively influence the health status of the cow. Olson et al. (1980) found that newborn calves under cold stress and hypothermia had significantly lower rates of absorption of Ig, particularly IgG and IgM, up to 15 hours after colostrum feeding. Therefore, in the present study, the greater concentration of Ig in colostrum from cows fed with açai oil was important to compensate for the reduced rate of absorption caused by the cold stress to the calves. Furthermore, calves under cold stress have reduced immunological systems, mainly because their bodies direct more energy to maintaining an adequate body temperature and consequently less is directed toward the immune system (Carroll et al. 2012). Taken together, the data suggest that greater absorption of immunoglobulins and antioxidants were important to maintain the immunological system in good conditions for newborn calves under cold stress conditions.

Table V. Serum levels of oxidants/antioxidant of calves born from cows fed with soybean (CONTROL) or açai (AÇAI) oil during close-up dry period (20.9 ± 2.6 days prior to calving).

Items[1]	Treatments[2]		P - values		
	CONTROL	AÇAI	Treat	Time	Treat × Time
SOD (U SOD/mg of protein)			0.88	0.01	0.87
0 h	3.65 (0.33)[A]	3.54 (0.30)[A]			
24 h	2.47 (0.33)[B]	2.56 (0.32)[B]			
120 h	2.38 (0.33)[B]	2.25 (0.32)[B]			
Average	2.83 (0.24)	2.78 (0.23)			
GST (U GST/mg of protein)			0.01	0.01	0.86
0 h	7.09 (0.71)[A]	8.60 (0.62)[A]			
24 h	4.04 (0.67)[B]	5.78 (0.65)[B]			
120 h	2.78 (0.67)[C]	4.90 (0.65)[B]			
Average	4.63 (0.27)[y]	6.43 (0.26)[x]			
GPx (U GPx/mg of protein)			0.09	0.68	0.48
0 h	0.22 (0.08)	0.45 (0.07)			
24 h	0.24 (0.09)	0.30 (0.07)			
120 h	0.21 (0.08)	0.39 (0.08)			
Average	0.22 (0.06)[y]	0.38 (0.05)[x]			
ROS (U DCF/mg of protein)			0.77	0.01	0.50
0 h	1.15 (0.18)[A]	1.36 (0.16)[A]			
24 h	0.70 (0.18)[B]	0.82 (0.17)[B]			
120 h	0.90 (0.18)[AB]	0.74 (0.17[B]			
Average	0.92 (0.13)	0.97 (0.11)			
ACAP (U.F./mg of protein)			0.11	0.13	0.83
0 h	0.29 (0.05)	0.20 (0.05)			
24 h	0.14 (0.05)	0.11 (0.05)			
120 h	0.28 (0.05)	0.20 (0.05)			
Average	0.24 (0.03)	0.17 (0.03)			
LPO (× 10^4 nmol/mL)			0.43	0.32	0.41
0 h	3.34 (1.98)	2.69 (1.67)			
24 h	4.30 (2.21)	3.32 (1.97)			
120 h	3.66 (1.98)	3.93 (1.80)			
Average	3.77 (1.33)	3.31 (1.26)			

[1]Superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), reactive oxygen species (ROS), antioxidant capacity against peroxyl radicals (ACAP) and lipid peroxidation (LPO).
[2]The treatments CONTROL or AÇAI represents the inclusion of 4.48% soybean or acai oil, respectively, for 20.9 ± 2.6 days prior to calving.
[x-y]Within a row, without a common superscript differ (*P* ≤ 0.05) or tends to differ (*P* ≤ 0.10).
[A-C]Within treatment, without a common superscript differ (*P* ≤ 0.05) or tends to differ (*P* ≤ 0.10).

CONCLUSION

The presence of açai oil in the feed of cows during the close-up dry period conferred immunological and antioxidant benefits. Cows fed with açai oil produced colostrum with greater concentrations of Ig and antioxidant capacity, effects that are desirable for rapid development of the immune system of newborn calves. These cows also produced colostrum with reduced lipoperoxidation and greater concentrations of IgA, both of which may have favored better absorption of total Ig in the calf intestine. Calves fed with colostrum from cows that received açai oil had greater serum protein levels accounted for by increased levels of heavy chain IgG, IgA, and α-lactalbumin in colostrum, all of which are involved in immune defenses. These data suggest that the addition of 4.48% açai oil in the close-up dry period of cows was an effective strategy

to improve colostrum quality, and consequently calves health.

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