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Acidified milk for feeding dairy calves in tropical raising systems

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ABSTRACT. The objective of this study was to evaluate the effect of milk acidification in tropical climate conditions on dairy calves' growth, health and selected blood metabolites. Thirty-two Holstein calves were blocked according to sex, birth date and weight, and distributed to the following treatments: 1. refrigerated milk kept at 5 °C (RM) or 2. acidified milk (with added lactic acid to a pH of 4.2) kept at ambient temperature (ACM). After birth, calves were fed colostrum and from the second day received 6 l/day of RM or ACM heated to 38 °C until weaning at day 56. Calves were individually housed with free access to water and starter diet. Feed intake and health problems were monitored daily; calves were weighed and measured weekly. Blood samples were collected weekly to evaluate the levels of metabolites. Feed intake, body weight and daily gain did not differ between treatments, but heart girth and wither height was higher for animals fed RM. The faecal score was lower for RM group, however in calves fed ACM it also did not suggest a diarrheal process (1.98). In addition, the first case of diarrhoea in calves fed ACM was later than in calves fed RM (15.4 vs 8.6 days, respectively; $P < 0.01$). So, the acidification of milk is an adequate method of preserving milk in tropical ambient temperatures. It resulted in health benefits to calves, delaying the first case of diarrhoea.

Introduction

According to a national survey, in Brazil about 44% of farmers use whole milk to feed calves, but when considering the use of waste milk this value is almost 80% (Santos and Bittar, 2015). Milk quality should be evaluated not only for its nutrient content, but also for its microbiological quality. Poor microbial quality may be responsible for the occurrence of diseases such as pneumonia and diarrhoea, impairing calves'performance, mainly in periods when the liquid diet is responsible for the most of energy intake (Aust et al., 2012). Microbial growth in milk tends to increase because of management failures, during milking or even during storage processes. The microbial growth may be a challenge especially in feeding programs with free access to the milk or in calf operations that do not have ways to refrigerate milk during storage (Moore et al., 2009).

Although the optimal growth conditions vary due to the presence of microorganisms, the nutritional composition of milk is ideal for the growth of many different pathogens. Temperature is also important for bacterial growth. In non-refrigerated milk (25–30 °C), bacterial growth is accelerated and causes milk degradation and alteration of the nutritional quality. However even during refrigeration,

psychotropic microorganism populations will increase, especially in Brazil where milk produced has high somatic cell score (SCC) during the summer season (Busanello et al., 2017). However, the main enterotoxigenic bacteria isolated from fresh milk and of high importance in the occurrence of neonatal calf diarrhoea such as *Escherichia coli* and *Salmonella* spp. are inhibited at low pH (Izzo et al., 2011).

The use of a pasteurizer is an alternative method for reducing the microbial load and feeding pasteurized milk reduces morbidity. However, this technique is not often financially feasible for smaller dairies because of high investment cost. Besides that, pasteurization does not prevent recontamination of milk, because it does not act as a sterilizator: some bacteria survive the process and can multiply rapidly in milk, mainly by management errors of the pasteurizer (Godden et al., 2005; Heinrichs and Jones, 2011). In addition, pasteurization reduces the population of lactic acid bacteria (LAB) that act as probiotics and are important for intestinal health (Deng et al., 2017).

Acidification of milk can be an alternative to these problems, because like refrigeration or pasteurization, it is a method to preserve the milk and maintain microbiological quality. The method is based on the addition of organic acids to reduce the milk pH and turn the environment unfavourable for the survival and growth of pathogenic microorganisms. According to Fallon and Harte (1988), this is the main advantage of milk acidification, although it is largely used in the *ad libitum* milk-feeding program (Todd et al., 2017), there are other benefits associated with the use of acidified milk. The modulation of digesta pH in the gastrointestinal tract (GIT) and the influence of the physiological processes of digestion may be a benefit of milk acidification for calves. The digestive tract pH reduction may be favourable for digestion processes through the formation of the abomasal clot, and concomitant decrease in the rate of multiplication of pathogens in the lower digestive tract (Fallon and Harte, 1988).

Even though the literature presented benefits for the feeding of acidified milk, there is no published data regarding its potential use in tropical production systems. Thus, the objective of this study was to compare the growth and feed intake, incidence of diarrhoea and metabolic parameters of Holstein calves fed with milk with different methods of conservation under high temperature climate conditions.

Material and methods

Experimental design and new born management

The experiment was conducted at Experimental Calf Facility of the 'Luiz de Queiroz' College of Agriculture, University of São Paulo, Brazil from January to August 2016 (average temperature 22.1 ± 3.6 °C, max 28.2 ± 2.9 °C and min 15.7 ± 4.4 °C and average relative humidity 77%). All procedures with calves were in accordance with the ethical standards of the University of Sao Paulo and Federal laws and were approved by the Institutional Animal Care and Use Committee. The study was conducted using 32 male and female Holstein newborn calves with average birth weight of 32.08 ± 1.75 kg. Immediately after birth, the calves were separated from their mothers, weighed and fed (100 g/kg body weight (BW)) colostrum within 6 h after birth, with a second meal given at 6–12 h after the first feeding. The colostrum quality was measured using a refractometer, and only high-quality colostrum (>23% Brix) was fed to the calves. Navels were treated with a 7% iodine solution for three consecutive days after birth. Calves were blocked according to sex, weight and date of birth and were randomly assigned to one of the following treatments: refrigerated milk (RM; n = 16) or acidified milk (ACM; n = 16).

For the evaluation of the passive immune transfer, blood samples were collected by venipuncture using vacuolated tubes without anticoagulants 48 h following colostrum feeding (VACUETTE do Brasil, Campinas, SP, Brazil). Average total serum protein of calves, measured using a digital handheld refractometer (ITREF 200, Instrutemp, São Paulo, SP, Brazil), was 6.0 g/dl, suggesting adequate passive immune transfer. After colostrum feeding, calves were fed 6 l/day of the corresponding liquid diet, divided in two meals (7:00 and 17:00).

Feeding and general management

Every two days, whole milk was collected from the University dairy farm, and half was refrigerated at 5 °C and the other half acidified to pH 4.2 by the addition of lactic acid (lactic acid 85%, Dynamics Química Contemporânea Ltda, Diadema, SP, Brazil) and maintained at room temperature. The pH was measured using a pH-meter (Tec-5 Tecnal Microprocessed pH meter Tecnal, Piracicaba, SP, Brazil) during the acidification, with inclusion of lactic acid until milk achieve the target pH of 4.2.

However, because during the storage period the pH tends to decrease to around 3.7–3.9, it was corrected again to pH 4.2 by adding whole milk to the mixture. This was done in an attempt to standardize the ACM pH fed to calves. The milk was acidified at least 6 h prior to feeding. Both RM and ACM were heated to 38–40 °C for feeding. The pre-weaning period lasted 56 days; however, gradual weaning started at day 52, with daily reduction of total volume to 4, 2, 1, 0.5 and 0 l at day 56 of age. Calves had free access to water and a commercial starter (Agroceres Multimix, Rio Claro, SP, Brazil). Starter intake was recorded daily and samples collected monthly for chemical analysis. Calves were housed in individual shelters (1.35 m height, 1 m width and 1.45 m depth), and contained with a chain belt attached to a thin chain, allowing an adequate walking area, but with no physical contact with other calf. Shelters were distributed in a trimmed grassy field.

Animal health

Faecal score was monitored daily by a veterinarian who did not know the treatment the calf was associated with, as described by Larson et al. (1977), regarding the fluidity of faeces: (1) normal and firm; (2) soft; (3) aqueous; (4) fluid. The diarrhoea episode was considered when the calves presented faecal score ≥ 3 for more than one day. Calves with a score ≥ 3 received oral rehydration solution. Calves rectal temperature was measured daily, using a digital thermometer, and the days with fever were considered when calves presented more than 39.4 °C.

Sampling and laboratory analysis

Calves were weighted weekly in a mechanical scale and corporal measurements were taken before the morning feeding, until weaning (56 day of age). Blood samples were collected on the same day of weighing, 2 h after the morning feeding *via* jugular vein puncture into evacuated tubes with and without anticoagulant (Vacuette of Brazil, Campinas, SP, Brazil). Samples were centrifuged (Universal 320R, Hettich, Tuttlinger, German) at 2000 *g*, for 20 min at 4 °C and plasma or serum were stored in a freezer (−26 °C) until subsequent analysis. Specific commercial enzymatic kits from Labtest Diagnóstica S.A. (Lagoa Santa, MG, Brazil) were used to analyse plasma glucose (ref. 85), total serum protein (ref. 99), lactate (ref. 116) using the Automatic System for Biochemistry-SBA200 (CELM, Barueri, SP, Brazil). The determination of beta-hydroxybutyric acid (BHB) was performed using the RANBUT enzyme kit (Ref.: RB1007; RANDOX Laboratories - Life Sciences Ltd., Crumlin, UK, imported by RANDOX Brasil Ltda., São Paulo, SP, Brazil) and the Automatic System for Biochemistry-SBA200 (CELM, Barueri, SP, Brazil). Haematocrit was determined with an aliquot of blood, collected from tube containing anticoagulant, using a microcentrifuge (Microspin, Model Spin 1000, USA) at 2000 *g* for 10 min and the measured in percentage.

Starter samples were collected monthly and ground through a 1-mm screen for analysis. Starter samples were analysed for dry matter (DM), ash and ether extract (EE) according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990). Crude protein (CP) was determined through combustion using FP-528 nitrogen analyser (Leco Corporation, St. Joseph, MI, USA). Neutral detergent fibre (NDF) was determined according to the method of Van Soest et al. (1991) using sodium sulphite and thermostable amylase. Total digestible nutrients (TDN) were calculated by the equations proposed by Weiss (1993) and non-fibre carbohydrate (NFC) according to the equation:

$$NFC = 100 - (CP + EE + NDFcp + ash),$$

where: CP – crude protein, EE – ether extract, NDFcp – NDF free of CP and ash.

Milk samples were taken weekly and were analysed for fat, protein and lactose by Fourier transform infrared spectroscopy (Lefier et al., 1996) and for SCC by flow cytometry (Clínica do Leite, Piracicaba, Brazil).

Samples of ACM were collected twice during the experimental period for determining lactic acid bacteria (LAB) and Enterobacteria concentrations, after serial dilution with distilled water (10^{-2} to 10^{-8}). The LAB was counted using Petrifilm plates (3M® do Brasil, São Paulo, Brasil), and inoculated plates were placed in closed jars containing anaerobic condition generator (ANAEROBAC; Probac Bacteriological Products of Brazil Ltda., São Paulo, Brazil) in a biochemical oxygen demand (BOD) incubator Model TE-402 (Tecnal, Piracicaba, SP, Brazil) at 32 ± 1 °C for 48 ± 3 h. For Enterobacteria counts, Petrifilm plates (3M® Brazil; Sumaré, SP, Brazil) were used and samples were incubated at 32.5 ± 1 °C in BOD incubator Model TE-402 for 24 ± 2 h. After the incubation period, counting proceeded by considering the areas with red colonies positive for LAB and red colonies with yellow and/or red colonies with gas bubbles, with or without yellow borders positive for Enterobacteria.

Statistical analysis

The experimental design was a randomized block, and calves were allocated to a total of 16 blocks according to their birth weight, date of birth and sex (6 female blocks and 10 male blocks). Differences in weight within the blocks were no more than 2 kg, while the age of the calves varied by a maximum of 15 days. The growth and intake data, blood metabolites and faecal score were analysed as time-repeated measures using the MIXED procedure of the SAS statistical package (version 5.0, SAS Institute Inc., Cary, NC, USA) for mixed models. The model included treatment, week (age of calves) and the interaction between treatment and week as fixed effects. The block effect was included in the model as a random effect. With week as a repeated measure (model 1), and the subject of the repeated measures used was animal (treatment). The covariance matrices were tested and defined according to the lowest value obtained for 'Akaike's Information Criterion Corrected' (AICC). The covariance structure Compound Symmetry (CS) was the best for starter intake, glucose and BHBA concentrations; while for lactate it was the Compound Symmetry Heterogeneous (CSH). For growth measures the Autoregressive (AR) structure was the best; while for body weight, faecal score and protein concentration the Autoregressive Heterogeneous (ARH (1)) was the best fit. For average daily gain and feed efficiency, the best covariance structure was Ante-dependence (ANTE (1)).

For data that were pooled to create a single measure, such as days for first diarrhoea, diarrhoea episodes/calf, days with faecal score 4 and days with fever, PROC MIXED of the SAS statistical package was used according to model 2. The model included treatment as fixed effect and block as random effect, and calf within treatment was included as a random effect. For all the responses variables the means were obtained through the LSMEANS command. The comparisons among the treatments were performed by the Tukey's test when there was significance in the analysis of variance. Significance was declared when $P \leq 0.05$ and a tendency when $0.05 < P < 0.10$.

$$Y_{ijk} = \mu + T_i + b_j + e_{ij} + S_k + (TS)_{ik} + e_{ijk},$$

where: Y_{ijk} – response variable, μ – general average, T_i – fixed effect of treatment, b_j – random block effect, e_{ij} – residual error (A), S_k – fixed age effect of calves (week of data collection/sample), $(TS)_{ik}$ – fixed effect of the treatment × age interaction, e_{ijk} – residual error (B).

$$Y_{ijk} = \mu + T_i + b_j + e_{ij},$$

where: Yijk – response variable, μ – general mean, T_i – fixed effect of treatment, b_j – random block effect, e_{ij} – residual error.

Results

Starter nutrient concentrations were within the levels recommended by the NRC (2001) for pre-weaning dairy calves (Table 1). Concentrations of milk fat, protein, lactose, total solids and SCC were within the normal range for dairy cows in Brazil (Table 1). Acidification was effective in reducing Enterobacteriaceae count in the first hour after acidification. The number of LAB was also reduced by the addition of lactic acid, with no viable LAB observed after 8 h (Table 1). Pre-acidification of whole milk contained 555 CFU/ml of Enterobacteriaceae and 4550 CFU/ml of LAB. When unrefrigerated whole milk was incubated, bacteria were too numerous to count, even during the first time point (1 and 2 h). No effect of treatment ($P > 0.05$) was detected for starter intake, average daily gain (ADG), birth and weaning weights, but starter intake and ADG increased ($P < 0.001$) in line with age (Figure 1). The body measures were not affected by treatments (Table 2), but increasing values were seen as calves got older.

Table 1. Starter concentrate and milk nutrient composition, somatic cell count (SCC) and growth of Enterobacteria and lactic acid bacteria (LAB) according to time after acidification

Starter concentrate							
DM, %	Protein, % DM	Ether extract, % DM	NDF, % DM	Ash, % DM	TDN, % DM		
87.4 ± 0.61	25.4 ± 0.45	5.9 ± 0.28	14.7 ± 0.61	8.6 ± 0.19	82.5 ± 0.57		
Milk							
Total solids, %	Protein, %	Fat, %	Lactose, %	SCC (x10^3/ml)			
12.83 ± 0.41	3.20 ± 0.01	4.15 ± 0.39	4.49 ± 0.01	496 ± 37.8			
		Acidified milk					
	Pre-acidification	0 h	1 h	2 h	4 h	8 h	12 h
Enterobacteria, CFU/ml	555	50	0	0	0	0	0
LAB, CFU/ml	4550	1575	1175	625	75	0	0

DM – dry matter, NDF – neutral detergent fibre, TDN – total digestible nutrients, CFU – colony forming units

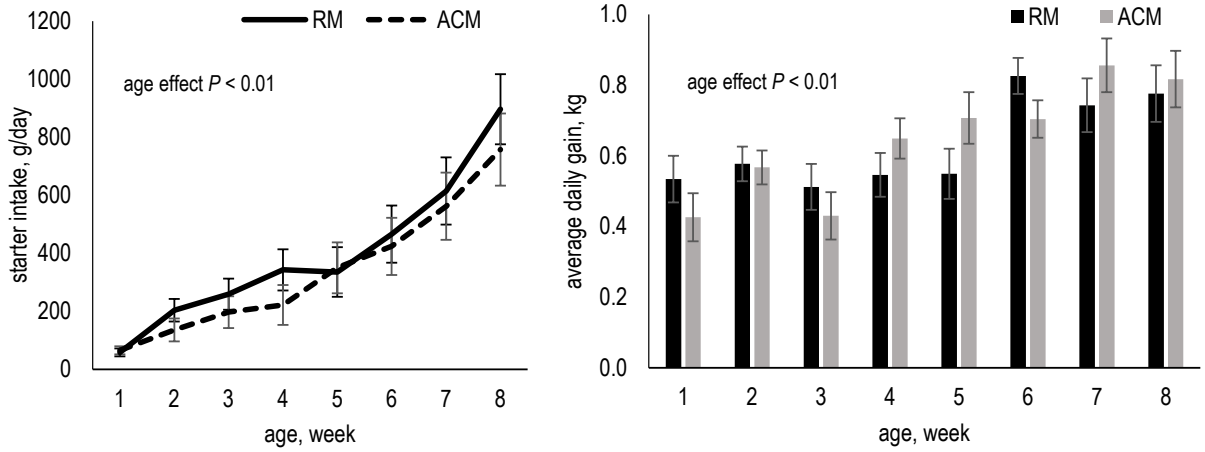

Figure 1. Starter intake and average daily gain of calves fed refrigerated (RM) or acidified (ACM) milk

Table 2. Performance of pre-weaned dairy calves fed either refrigerated (RM) or acidified (ACM) milk

Indices	Treatment		SEM	*P*-value[1]		
	RM	ACM		T	A	T×A
Intake						
starter, g/day	393	336	42	0.25	0.01	0.73
Weight, kg						
at birth	31.5	31.5	1.69	0.97	-	-
at weaning	67.4	68.2	1.98	0.61	-	-
Average daily gain, kg/day	630	640	30.0	0.57	0.01	0.17
Feed efficiency						
body measurements, cm						
heart girth	85.1	84.3	1.19	0.30	0.001	0.27
hip width	23.5	22.3	1.01	0.40	0.07	0.46
withers height	79.9	79.6	1.00	0.65	0.01	0.67

[1] T – treatment effect, A – age effect, T×A – treatment × age interaction effect ($P < 0.05$); SEM – standard error of the mean

Faecal score was higher (more fluid faeces) for calves fed ACM in comparison to those fed RM (Table 3; $P < 0.05$), but was not characterized as diarrhoea since the score was lower than 3. Haematocrit was also not affected ($P < 0.05$) suggesting that even with higher faecal scores calves were not experiencing clinical diarrhoea. There was an interaction of treatment and age ($P < 0.05$) for faecal score because calves fed RM had higher faecal scores at 2 week of age (Figure 2). Days with score 4 (severe diarrhoea) and days with fever were not affected by liquid diet ($P > 0.05$), however there was a trend for higher number of episodes/calf ($P < 0.09$) for calves fed ACM.

Plasma glucose concentration tended to be higher for calves fed RM in comparison to those fed ACM ($P < 0.07$; Table 4), and there was a tendency for glucose concentration to decrease ($P < 0.07$) along with the age. Concentration of BHB did not differ between the treatments, but increased with age ($P < 0.01$; Figure 3). Total protein and lactate concentrations (Table 4) did not differ between

Table 3. Diarrhoea and health of calves fed refrigerated (RM) or acidified (ACM) milk

Indices	Treatment		SEM	*P*-value[1]		
	RM	ACM		T	A	T×A
Haematocrit	23.0	23.7	0.57	0.34	0.21	0.65
Faecal score	1.80[a]	1.99[b]	0.05	0.02	< 0.01	0.04
First diarrhoea, day	8.6[b]	15.4[a]	1.7	0.010	-	-
Number episodes/calf	1.8	2.6	0.3	0.085	-	-
Days with faecal score 4	1.6	1.5	0.5	0.858	-	-
Days with fever	0.75	0.25	0.28	0.230	-	-

[1]T – treatment effect, A – age effect, T×A – treatment × age interaction effect ($P < 0.05$); SEM – standard error of the mean; [ab] – values in the same row with different superscripts are significantly different at $P < 0.05$

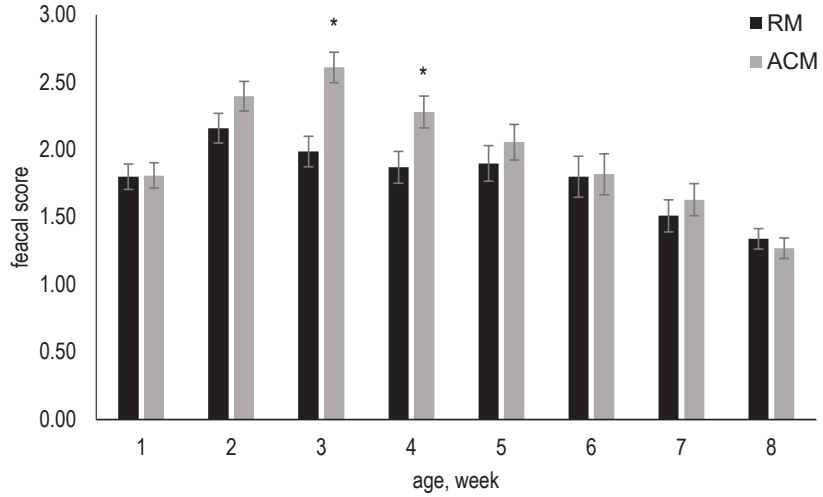

Figure 2. Faecal score of calves fed refrigerated (RM) or acidified (ACM) milk. * – denotes difference between treatments in that particular week of age; age effect $P < 0.01$, age × treatment effect $P < 0.04$

Table 4. Blood metabolites of calves fed refrigerated (RM) or acidified (ACM) milk

Indices	Treatment		SEM	*P*-value[1]		
	RM	ACM		T	A	T×A
Glucose, mg/dl	126	119	2.8	0.07	0.07	0.24
BHB, mmol/l	0.160	0.157	0.007	0.77	<0.01	0.36
Total serum protein, g/dl	6.64	6.60	0.13	0.74	<0.01	0.14
Lactate, mg/dl	11.21	11.43	0.57	0.78	<0.01	0.13

[1]T – treatment effect, A – age effect, T×A – treatment × age interaction effect ($P < 0.05$); SEM – standard error of the mean

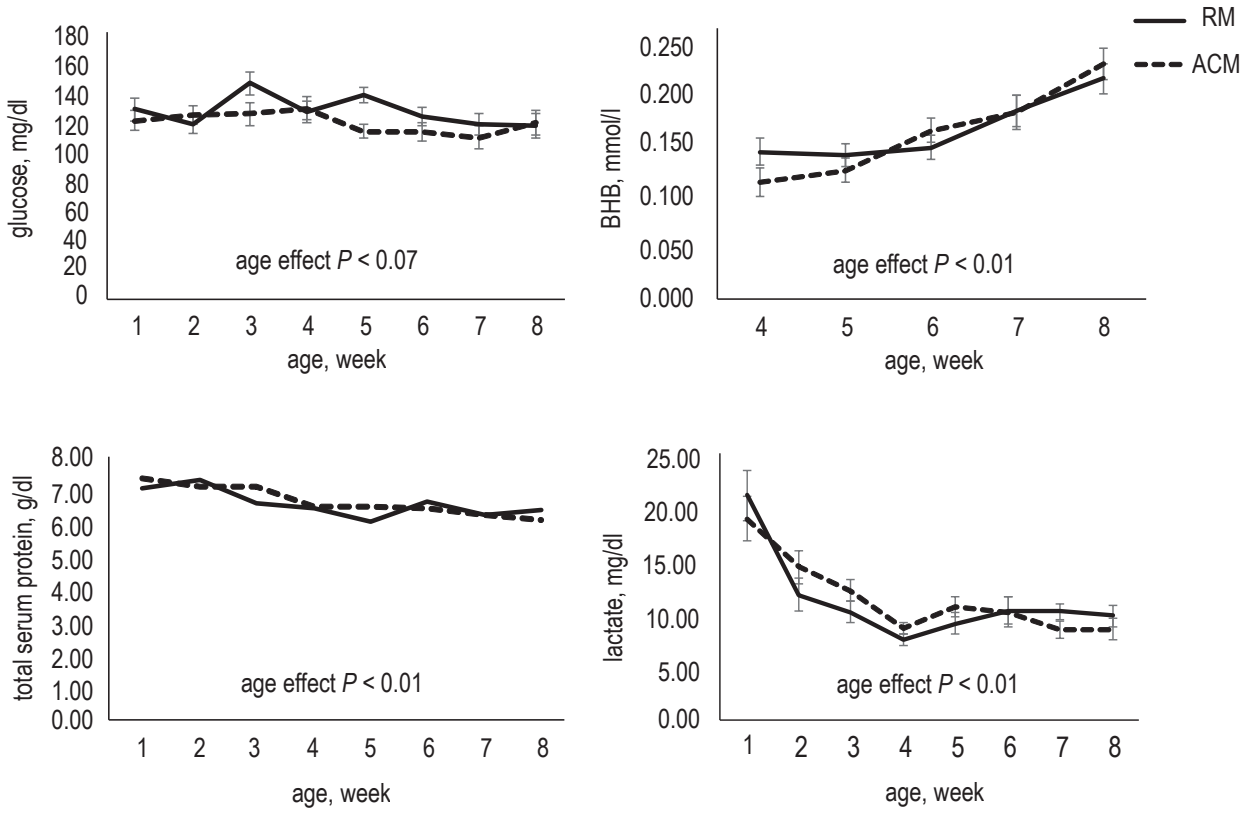

Figure 3. Glucose, β-hydroxybutyric acid (BHB), total protein and lactate blood concentrations of calves fed refrigerated (RM) or acidified (ACM) milk

treatments ($P > 0.05$), but decreased ($P < 0.01$) values as calves aged (Figure 3). No interaction of treatment and age ($P > 0.05$) was observed for all measured blood metabolites.

Discussion

Although there was a decrease of Enterobacteria in ACM, the decrease in LAB, with no growth after 8 h of acidification, may indicate a disadvantage of adding lactic acid to milk for feeding calves. However, regardless of decreased concentrations of LAB, the lower pH favours intestinal microbial colonization with beneficial microorganisms. In general, LAB are probiotic microorganisms such as those from the genus *Bifidobacterium* and species such as *Lactobacillus acidophilus* (Deng et al., 2017). These microorganisms synthesize bacteriocins, which are antimicrobial peptides that act competitively against pathogenic bacteria and therefore function as an important constituent of the microbial defence system (Nes et al., 2007). However, in this study it is possible to observe that the LAB population tends to reduce after acidification.

According to the NRC (2001), calves fed 6 l/day of milk have a metabolizable energy and protein intake to support a daily gain of about 670 g, which agrees with that observed in the present study. Calves were fed at a rate of more than 18% of birth weight, characteristic of an intensive liquid feeding program (≥15%; de Paula et al., 2017). Feeding more than 6 l/day of milk may decrease starter intake, which may difficult the weaning process of calves without decreasing performance. Considering the 12.83% of total solids, calves were fed an equivalent of 770 g/day DM. According to Gelsinger et al. (2016) when milk DM intake exceeds 800 g/day, there may be a stronger effect on starter intake. The adoption of gradual weaning process also aid calves in adjusting intake and keep ADG after weaning (de Paula et al., 2017). According to Quigley et al. (1991) calves should consume more than 700 g/day of starter to be weaned without decreasing performance post-weaning. Since then, the recommendation has increased to 1.5 kg/day of starter intake (Stamey et al., 2012). In the present study, treatment had no effect on starter intake at weaning, suggesting that calves would maintain ADG after that. Calves began to consume starter in the first week of age and were able to double their birth weight at the milk-feeding phase, as suggested by the literature (Van Amburgh and Drackley, 2005).

Acidification had no effect on calves' growth, mostly because there were no effects on feed intake. Zhang et al. (2016) suggested that the pH reduction in milk could help abomasal digestion, promoting the formation of clots, which improves the digestibility of nutrients favouring the calves growth rate. Zou et al. (2017) reported improved starter intake when ACM was fed *ad libitum*. However, Todd et al. (2017) found no beneficial growth effect of feeding milk acidified to 4–4.5 pH in comparison to whole milk in a restricted feeding program, which was also observed in the present study with ACM. Fallon and Harte (1988) observed higher daily weight gain in calves fed acidified milk replacer (pH 5.8) than in calves fed non-acidified milk replacer. Improvement in growth of calves fed ACM is usually due to improved digestion of milk or increased starter intake (Zhang et al., 2016; Todd et al., 2017) in addition to reducing microbial growth (Anderson, 2008). However, in this study similar performance should be considered as an advantage for ACM since no refrigeration was required to maintain milk microbial quality, and there was no damage to the animal health, resulting in calves with similar weaning weight.

Because there were no differences in starter intake and milk feeding was fixed, there were no differences in average daily gain or structural growth between treatments. Structural growth is highly correlated with nutrient intake provided by milk and starter. In general, structural growth deficiencies are correlated with low-protein intake (Deng et al., 2017). Because calves were fed milk containing the same percentage of protein and there were no differences in liquid and solid diet intake, as well as in total serum protein. Todd et al. (2017) reported that calves fed ACM replacer free-choice had greater pre-weaning structural growth as compared to those fed restricted volumes, suggesting that effects were more likely due to nutrient intake rather than the acidification per se. However, in some studies it was found that body measurements of calves were not affected by feeding ACM or ACM replacer (Li et al., 2019; Sun et al., 2019).

According to Anderson (2008), acidification is recommended for farms that provide high volumes of milk that are available for an *ad libitum* intake. However, it also may suit farms that have a high incidence of diarrhoea due to poor milk microbiological quality. Some farms do not have a way to refrigerate waste milk from the last milking of the day until the next morning feeding, which results in the increased rates of diarrhoea. In the present study, calves were

fed ACM kept at room temperature with no effect on calves growth, however, faecal scores increased during weeks 2 and 3. Even though the faeces were more fluid for calves fed ACM, the first case of diarrhoea occurred later (15.4 vs 8.6 day of age), which agrees with the observations of Todd et al. (2017): first diarrhoea occurrence at 9.8 and 5.5 day for calves receiving diet with and without acidification, respectively. Calves are born with an immature immune system and agammaglobulinemic, depending on colostrum intake. Over the days and with exposure to antigens the system tends to develop gradually, which makes calves less susceptible to more severe infections (Chase et al., 2008). Calves fed ACM presented a higher faecal score (1.80 vs 1.99) and although the first cases occurred when calves were older, there was a tendency for higher episodes of diarrhoea/calf. On the other hand, even though faeces were more fluid, which may suggest a decreased nutrient availability, there were no differences in ADG and final body weight. Haematocrit values and days with fever also suggest that calves fed ACM were healthy, but with more fluid faeces.

Concentrations of BHB increased with advancing age and starter intake as expected for calves with a developing rumen. According to Quigley et al. (1991), BHB concentrations increases with increasing starter intake and, consequently, the onset of metabolism of butyrate to ketone bodies by the developing ruminal epithelium. Plasma glucose is closely correlated with the presence and absorption of carbohydrates from the diet, especially lactose. Plasma glucose tended to decrease during the experimental period in ACM group, suggesting that acidification can reduce the lactose content of milk. Glucose concentrations normally decrease as the animal begins to use fermentation products such as butyrate as a source of energy (Suarez-Mena et al., 2017).

Total protein is the simplest way of assessing the animal's protein status (Payne and Payne, 1987). Because of this, its decrease translates into diet deficiency or inability to absorb protein, due to diarrhoea, for example (Kaneko, 1997), or because of decreased colostrum proteins absorbed by the newborn. Plasma protein concentrations observed in this study are within the normal reference values (6–8.5 mg/dl) for Holstein calves (Luca and Reis, 2001). Lactate, on the other hand, translates to the high concentration of easily fermented carbohydrates in the diet of calves. These compounds are quickly degraded by ruminal bacteria to produce lactate, with a small portion being metabolized by the liver and the surplus remaining in the circulation. However, plasma concentrations remained within the normal reference values (between 6 and 22 mg/dl) (Kasari and Naylor, 1984), emphasizing the quality of the diet in promoting a healthy ruminal environment.

Conclusions

Acidification of whole milk proved to be a good alternative for milk conservation, even in tropical climate conditions. It did not negatively affect the animal growth in comparison to the refrigerated milk. Feeding calves acidified milk adjourns the first incidence of diarrhoea, and although the faecal scores suggest that faeces were more fluid, calves were healthy, as did not present fever and had similar final weight.

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References

Anderson N.G., 2008. Experiences with free-access acidified-milk feeding in Ontario. In: Proceedings of Annual Meeting of American Association of Bovine Practitioners. Charlotte, NC (USA), No. 41, pp. 12−324

AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Edition. Arlington, VA (USA)

Aust V., Knappstein K., Kunz H.J., Kaspar H., Wallmann J., Kaske M., 2012. Feeding untreated and pasteurized waste milk and bulk milk to calves: Effects on calf performance, health status and antibiotic resistance of faecal bacteria. J. Anim. Physiol. Anim. Nutr. 97, 1091−1103, https://doi.org/10.1111/jpn.12019

Busanello M., Rossi R.S., Cassoli L.D., Pantoja J.C.F., Machado P.F., 2017. Estimation of prevalence and incidence of subclinical mastitis in a large population of Brazilian dairy herds. J. Dairy Sci. 100, 6545−36553, https://doi.org/10.3168/jds.2016-12042

Chase C.C.L., Hurley D.J., Reber A.J., 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. N. Am. Food Anim. Pract. 24, 87−104, https://doi.org/10.1016/j.cvfa.2007.11.001

de Paula M.R., Oltramari C.E., Silva J.T., Gallo M.P.C., Mourão G.B., Bittar C.M.M., 2017. Intensive liquid feeding of dairy calves with a medium crude protein milk replacer: Effects on performance, rumen, and blood parameters. J. Dairy Sci. 100, 4448−4456, https://doi.org/10.3168/jds.2016-10859

Deng Y.F., Wang Y.J., Zou Y. et al., 2017. Influence of dairy by-product waste milk on the microbiomes of different gastrointestinal tract components in pre-weaned dairy calves. Sci. Rep. 7, 42689, https://doi.org/10.1038/srep42689

Fallon J.R., Harte F.J., 1988. Effect of normal or acidified milk replacer offered ad libitum on calf performance. Irish J. Agric. Food Res. 27, 123−130, https://jstor.org/stable/25556215

Gelsinger S.L., Heinrichs A.J., Jones C.M., 2016. A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. J. Dairy Sci. 99, 6206−6214, https://doi.org/10.3168/jds.2015-10744

Godden S.M., Fetrow J.P., Feirtag J.M. et al., 2005. Economic analysis of feeding pasteurized nonsaleable milk versus conventional milk replacer to dairy calves. J. Am. Vet. Med. A. 9, 1547−1554, https://doi.org/10.2460/javma.2005.226.1547

Heinrichs J., Jones C., 2011. Composition and hygiene of colostrum on modern Pennsylvania dairy farms. Penn. State Dairy An. Sci. 171, 1−36

Izzo M.M., Kirkland P., Mohler V., Perkins N., Gunn A., House J., 2011. Prevalence of major enteric pathogens in Australian dairy calves with diarrhea. Aust. Vet. J. 89, 167−173, https://doi.org/10.1111/j.1751-0813.2011.00692.x

Kaneko J.J., 1997. Serum proteins and the dysproteinemias. In: J.J. Kaneko, J.W. Harvey, M.L. Bruss (Editors). Clinical Biochemistry of Domestic Animals. San Diego Academic Press. San Diego, CA (USA), pp.117−138, https://doi.org/10.1016/B978-012396305-5/50006-3

Kasari T.R., Naylor J.M., 1984. Metabolic acidosis without clinical signs of dehydration in young calves. Can. Vet. J. 25, 394−399

Larson L.L., Owen F.G., Albright J.L., Appleman R.D., Lamb R.C., Muller L.D., 1977 Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60, 989−991, https://doi.org/10.3168/jds.S0022-0302(77)83975-1

Lefier D., Grappin R., Pochet P., 1996. Determination of fat, protein, and lactose in raw milk by Fourier Transform Infrared Spectroscopy and by analysis with a conventional filter-based milk analyzer. J. AOAC Int. 79, 711−717, https://doi.org/10.1093/jaoac/79.3.711

Li L., Qu J., Xin X., Yin S., Qu Y., 2019. Comparison of reconstituted, acidified reconstituted milk or acidified fresh milk on growth performance, diarrhea rate, and hematological parameters in preweaning dairy calves. Animals 9, 778, https://doi.org/10.3390/ani9100778

Luca G.C., Reis B.F., 2001. Spectrophotometry of total proteins in bovine blood plasma by flow analysis (in Portuguese). Sci. Agric. 59, 251−256, https://doi.org/10.1590/S0103-90162002000200007

Moore D.A., Taylor J., Hartman M.L., Sischo W.M., 2009. Quality assessments of waste milk at a calf ranch. J. Dairy Sci. 92, 3503−3509, https://doi.org/10.3168/jds.2008-1623

Nes I.F., Yoon S.S., Diep D.B., 2007. Ribosomally synthesized antimicrobial peptides (bacteriocins) in lactic acid bacteria: a review. Food Sci. Biotechnol. 16, 675−690

NRC (National Research Council), 2001. Nutrient Requirements of Dairy Cattle. The National Academies Press. Washington, DC (USA), https://doi.org/10.17226/9825

Payne J.M., Payne S., 1987. The Metabolic Profile Test. Oxford University Press. Oxford (UK)

Quigley J.D. III, Smith Z.P., Heitmann R.N., 1991. Changes in plasma volatile fatty acids in response to weaning and feed intake in young calves. J. Dairy Sci. 74, 258−263, https://doi.org/10.3168/jds.S0022-0302(91)78168-X

Santos G., Bittar C.M.M., 2015. A survey of dairy calf management practices in some producing regions in Brazil. Rev. Bras. Zootec. 44, 361−370, https://doi.org/10.1590/S1806-92902015001000004

Stamey J.A., Janovick N.A., Kertz A.F., Drackley J.K., 2012. Influence of starter protein content on growth of dairy calves in an enhanced early nutrition program. J. Dairy Sci. 95, 3327−3336, https://doi.org/10.3168/jds.2011-5107

Suarez-Mena F.X., Hu W., Dennis T.S., Hill T.M., Schlotterbeck R.L., 2017. β-Hydroxybutyrate (BHB) and glucose concentrations in the blood of dairy calves as influenced by age, vaccination stress, weaning, and starter intake including evaluation of BHB and glucose markers of starter intake. J. Dairy Sci. 100, 2614−2624, https://doi.org/10.3168/jds.2016-12181

Sun Y., Li J., Meng Q., Wu D., Xu M., 2019. Effects of butyric acid supplementation of acidified milk on digestive function and weaning stress of cattle calves. Livest. Sci. 225, 78−84, https://doi.org/10.1016/j.livsci.2019.04.021

Todd C.G., Leslie K.E., Millman S.T., Bielmann V., Anderson N.G., Sargeant J.M., Devries T.J., 2017. Clinical trial on the effects of a free-access acidified milk replacer feeding program on the health and growth of dairy replacement heifers and veal calves. J. Dairy Sci. 100, 713−725, https://doi.org/10.3168/jds.2016-11401

Van Amburgh M., Drackley J.K., 2005. Current perspectives on the energy and protein requirements of the pre-weaned calf. In: P.C. Garnsworth (Editor). Calf and heifer rearing: principles of rearing the modern dairy heifer from calf to calving. Nottingham University Press. Nottingham (UK)

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583−3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Weiss W.P., 1993. Predicting energy values of feeds. In: Symposium: Prevailing concepts in energy utilization by ruminants. J. Dairy Sci. 76, 1802−1811, https://doi.org/10.3168/jds.S0022-0302(93)77512-8

Zhang R., Zhou M., Tu Y., Zhang N.F., Deng K.D., Ma T., Diao Q.Y., 2016. Effect of oral administration of probiotics on growth performance, apparent nutrient digestibility and stress-related indicators in Holstein calves. J. Dairy Sci. 100, 33−38, https://doi.org/10.1111/jpn.12338

Zou Y., Wang Y., Deng Y., Cao Z., Li S., Wang J., 2017. Effects of feeding untreated, pasteurized and acidified waste milk and bunk tank milk on the performance, serum metabolic profiles, immunity, and intestinal development in Holstein calves. J. Anim. Sci. Biotechnol. 8, 53−64, https://doi.org/10.1186/s40104-017-0182-4