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Research Article

Evaluation of the Raw Milk Quality Produced in Semiarid Areas from the Alagoas, Brazil: The influence of Vegetation Index and Bovine Thermal Comfort Index

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Abstract

The aim of this study was to evaluate the quality of the raw milk produced in the semiarid region of the Alagoas state, Brazil being influenced by bovine thermal control index and vegetation index. The raw milk was collected after the dairy cow of 12 small rural areas (semi-arid) of Alagoas state during four seasons. Fifty-eight collected raw milk samples were subjected to examine microbiologically for coliforms, Escherichia coli and coagulase-positive Staphylococcus. Only 02 rural associations presented low levels of microbiological contamination, which were located in areas of climatic conditions and parameters of Bovine Thermal Comfort Index (BCTI) and vegetation index favorable, but 10 rural associations presented high counting of coliforms at 45°C, Escherichia coli. The BTCI and Vegetation Index have shown to influence growth of coliforms at 45°C and Escherichia coli with high incidence during summer condition. The BCTI and vegetation index had influenced the coagulase-positive Staphylococcus count especially during the end of summer and start of the autumn seasons.

1. Introduction

Milk is considered to be a complete foods and nutritional key terms for the human diet and constitutes an excellent substrate for the development of a wide variety of microorganisms, including pathogens. The milk and dairy products show a frequent concern for technical and authorities related to the area health, particularly the risk of transmission of microorganisms related to Foodborne Disease (FD) outbreaks (Silva et al., 2007).

Microflora species present predominantly in raw milk are usually the of lactic acid bacteria (*Lactococcus, Lactobacillusspp., Leuconostoc, Enterococcus or Streptococcus* spp.), *Pseudomonas* spp., bacteria belonging to the family *Micrococcaceae (Micrococcus* and *Staphylococcus* spp.) and yeasts. Other microbial groups also present including: *Bacillus, Clostridium, Listeria* spp., Enterobacteriaceae species such as *Acinetobacter, Alcaligenes, Flavobacterium* and *Aeromonas, Arthrobacter, Corynebacterium, Brevibacterium* e *Propionibacterium* (Lafarge et al., 2004).

There is no specific regulation in Brazil on the microbiological quality of raw milk for the manufacture of specific dairy products (Pinto, 2006), the trade of this product represents an important alternative, because of there is an established demand for unpasteurized raw milk in Brazil, despite the illegality (Nero et al., 2004). Data about the Foodborne Diseases (FD) caused by raw milk consumption are inconsistent and little information is available on the occurrence of pathogens in the Brazilian raw milk (Nero et al., 2004). When referring to productive factors biometeorology is the science that deals with the effects of environmental stress that limit a great animal production and environmental management strategies to reduce stress and enhance the production (productive and reproductive performance) and health (Baccari Júnior, 1998).

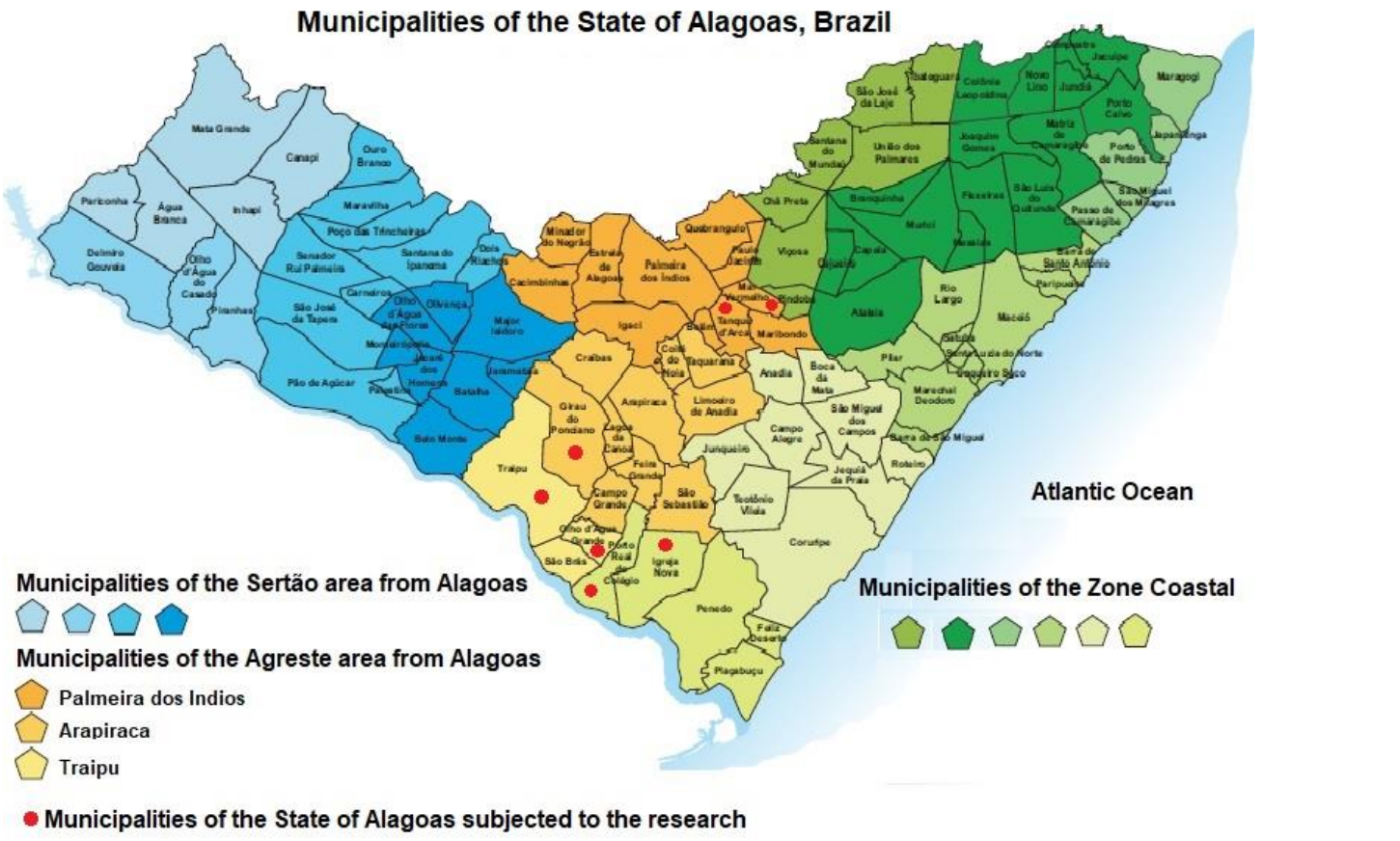

Figure 1. Map of the State of Alagoas and municipalities highlighting in red the locations of associations of milk producers subjected to the research
(Source: http://www.wikialagoas.al.org.br/index.php/Arquivo:Mapa_de_alagoas_por_meso_e_micro.png)

Table 1. The number of samples taken in rural associations receiving of raw milk by Municipalities between July 2014 and April 2015**.**

Municipalities of Alagoas	Rural Association	Number of samples taken by the municipality, between July 2014 and April 2015					Total Samples
		July 2014	September 2014	November 2014	January 2015	April 2015	
Tanque D'arca	01	01	01	01	01	-	04
Mar Vermelho	01	01	01	01	01	-	04
Girau do Ponciano	01	01	01	01	01	01	05
Traipu 1	03	03	03	03	03	03	15
Olho D'água Grande	01	01	01	01	01	01	05
Igreja Nova 1	03	03	03	03	03	03	15
Porto Real do Colégio	02	02	02	02	02	02	10

The bovine herd is submitted to adverse conditions at the north-east of Brazil (Santos, 2009). This researcher mentions the importance both for the animal, as for pasture production, climatic factors such as humidity, temperature, rainfall, frost, among others. The fragility of bovine cattle compromise the sustainable development and the milk production network in semi-arid regions of northeastern from Brazil, and consequently the supply of milk with low quality (Carvalho Filho, 2011).

Weather conditions directly or indirectly influence the development of animal behavior: how to forage, normal body functions, animal lifestyle and some diseases that predominate determinate region. In dry conditions, the

forages are impaired in the nutrient quality, interfering with the decrease in milk production and body weight. Availability of insufficient water offering to animals is evident in causing discomfort, body dehydration and illness especially in the regions with warmer temperatures (Ayode, 2002).

The severe hot weather conditions can be estimate by the climate of a region, considering the climatic factors such as temperature, relative humidity, solar radiation and wind speed, combined in a single variable, the effective temperature. Stress coming from high temperatures and humidity may result in increased susceptibility to intra-mammary infections, as well as increase the number of pathogens to which the cows are exposed (Ricci, 2013).

This study aimed to evaluate the quality of the raw milk produced in the semi-arid areas of the State of Alagoas-Brazil and its relation with the influence of the vegetation index and bovine thermal comfort index.

2. Material and Methods

2.1 Sample planning and collection of the samples

This study was conducted in seven (07) municipalities of the State of Alagoas. The study was also based on the previous studies for the quality evaluating of the raw milk in critical areas the State of Alagoas, Brazil (Soares, 2014; Santos, 2014). The identity of Rural Associations was assigned from A1 to A12 identification codes. A total of twelve associations of farmers and milk producers, (based on the market of the Milk Program), of the municipalities of Tanque D'arca (A1), Mar Vermelho (A2), Girau do Ponciano (A3), Traipu (A4, A5, A6), Olho D'água Grande (A7), Igreja Nova (A8, A9, A10), and Porto Real do Colégio (A11, A12) were evaluated (Figure 1).

Samples of refrigerated raw milk were collected in small rural associations of milk producers located in seven (07) municipalities from the State of Alagoas between the months of July/ 2014 and April/ 2015. The first collection was in Jul/ 2014; the second collection in Sep/ 2014; third collection in Nov/ 2014; fourth collection in Jan/ 2015 and the fifth collecting in April/ 2015. The collections planning was to run on one (01) year, performing on alternate months at maximum intervals of three months between each collection contemplating the four seasons (winter, summer, fall, and spring) (Table 1).

The collection of samples came directly from refrigerated bulk tanks of the Rural Associations, which was storage in controlled temperature at 4 °C. All samples were stored in autoclaved and sterile bottles. Then, these samples were transported under refrigeration in cool boxes with ice foam sheets. The microbiological assays were performed at the Laboratory of food microbiological quality control of the College of Nutrition from the Federal University of Alagoas.

2.3. Microbiological Analysis

The samples were subjected to determination of the Most Probable Number (MPN/mL) for Coliform at 45 °C analysis, *Escherichia coli* analysis and *coagulase-positive Staphylococcus aureus* analysis as follows the method of American Public Health Association (APHA) – Compendium of Methods for the Microbiological Examination of Foods and Standard Methods for the Examination of Dairy Products (Silva, 2007).

2.4 Meteorological Weather Variables during the Milk Collection

2.4.1 Description Modeled Data (Reanalysis)

The weather data were extracted from National Institute of Meteorology from the Brazilian government (INMET) in the august of 2016. The modelled data were obtained from the base of *European Centre for Medium-Range WeatherForecasts* (ECMWF) available at website <www.ecmwf.int>, being one of the world centre responsible for distributing data throughout the world. The modelled data are based on interpolation methods, as an alternative to minimize the gaps caused by the lack of meteorological stations in some regions of the globe, making estimates of various meteorological variables. The extracted data are part of a product running on ECMWF, the ERA-INTERIM, to which it has an early set of data January 01, 1979 until the 07/31/2015 (Dee et al., 2011). These data are provided in CAN synoptic times (6 in 6 hours) OR ALSO How Forecast (3 in 3 hours) furthermore, the same set has a spatial resolution of 0.125º x 0.125º (1º corresponds approximately 111 km), that is, generating a rough grid 14km x 14km. In this extraction, were used Daily Data of 10 meteorological variables, between the period of July 1, 2014, to April 30, 2015. Some of the data used needed to be converted, for example: was converted Kelvin temperature (K) Celsius (°C).

Table 2. The municipalities studied and based on the approximation of grid points.

Municipalities	Latitude (ºS)	Longitude (ºW)
Girau do Ponciano	-9,875	-36,875
Igreja Nova	-10,125	-36,625
Mar Vermelho	-10,125	-36,625
Olho D'água Grande	-10	-36,75
Porto Real do Colégio	-10,125	-36,75
Tanque D'Arca	-9,625	-36,375
Traipu	-10	-36,875

2.4.2 Determination of Precipitation Data

For precipitation data, seven grid points were used and these points corresponded to the location closest to each municipality used in this study. The area comprised between latitudes 9.5ºS-10.25ºS and longitudes 36.375º W-37.125ºW. Table 2 demonstrates the geographic coordinates corresponding to each one of them.

As for the rainfall data, they came from the *Tropical Rainfall Measuring Mission* (TRMM) of version 7 of the 3B42 algorithm, which is part of the joint mission between the *National Aeronautics Space Administration* (NASA) and *Japan Aerospace Exploration* (JAXA). To study rainfall for research related to weather and climate (Huffman et al., 2007). These data are obtained from satellites by microwave extraction from three sensors: *Visible and Infrared Scanner* (VIRS*), Lightning Imaging Sensor* (LIS) and *Clouds and the Earth's Radiant Energy System* (CERES), and have temporal resolution at each 3 hours and a space of 0.5º x0.5º (approximately 67.5 km x 67.5 km). In this work, daily data were used for the period between July 2014 and April 2015.

2.5. Bovine Thermal Comfort Index (BTCI) and Vegetation Index

The Bovine Thermal Comfort Index (BTCI) and Vegetation Index were collected through National Institute of Meteorology (INMET, 2016), from Brazilian government, observing the period between July 2014 and July 2015. The satellite data of the municipality of Propriá-Sergipe was used as approaching for the axis of the Traipu, Porto Real do Colégio, Igreja Nova, Girau do Ponciano and Olho D'Água Grande municipalities of the State of Alagoas with adjacent areas. The data of the municipality of Palmeira dos Índios-Alagoas was used as approaching for the axis of the Mar Vermelho and Tanque D´Arca municipalities.

2.6 Statistical analysis

All raw milk samples were sampling in sufficient quantities for three replicate analysis. The Microbiological analysis were performed in three replicates.

3. Results and Discussion

Microbiological data showed a significant count of Coliforms at 45 ºC, *Escherichia coli and* coagulase-positive *Staphylococcus* for the raw milk samples from some semiarid areas of the State of Alagoas, Brazil. There is low contamination for the Tanque D´Arca and Mar Vermelho municipalities, which were located in the semiarid area of hill and mountains close to Palmeira dos Índios meteorological station. But for the areas located near to the São Francisco River, named as (Area from the Baixo São Francisco) and located to the Propiá meteorological station, the contamination parameters were very high was and dependent on the season of the year and climatic transition period, which were presented and discussed.

3.1 Microbial Detection

3.1.1 Coliforms at 45 ºC and Escherichia coli Counting

The results obtained in this study demonstrate that the Coliform at 45 ºC counts was at the range from < 3.0 the > 1,100 NMP/mL and the presence of *Escherichia coli* occurred in 86.2% of samples of raw milk (Table 3). The rural associations of Tanque D'Arca and Mar Vermelho presented low levels of coliforms at 45 ºC in relation to others rural associations located at the area from the "Baixo São Francisco Area".

The microbiological assays for coliforms at 45 ºC showed the scenario microbiological of these associations. The Tanque D'Arca and Mar Vermelho rural associations had the lowest contamination index in the raw milk analyzed, although the presence of *Escherichia coli* was confirmed (Figure 2). The Traipu 1, Olho D´água Grande and Igreja Nova 1 rural associations portrayed a moderate level of contamination with great variation in the fourth and fifth collections. These rural associations pointed out for the most part counts between <3.0 MPN/mL and ≥1,100 MPN/mL and in all of them the existence of *Escherichia coli* was detected (Table 3).

The investigation of Coliforms 45 ºC and *Escherichia coli* in food products are indicators of the lack of hygienic

Table 3. Distribution of the counting for Coliform at 45 ºC and *Escherichia coli* in 58 samples of refrigerated raw milk from different regions of Alagoas

Rural associations	**Counts of coliforms at 45 ºC (MPN/mL) / *Escherichia coli***				
	1st collection	**2nd collection**	**3rd collection**	**4th collection**	**5th collection**
Tanque D'arca	7.4/(+)	120/(+)	6.1/(+)	23/(+)	-
Mar Vermelho	9.2/(+)	75/(+)	3.0/(+)	9.2/(+)	-
Girau do Ponciano	1,100/(+)	>1,100/(+)	93/(+)	>1,100/(+)	>1,100/(+)
Traipu 1	43/(+)	23/(+)	< 3.0/(-)	3.6/(-)	1,100/(+)
Traipu 2	43/(+)	>1,100/(+)	35/(+)	>1,100/(+)	>1,100/(+)
Traipu 3	< 3.0/(-)	210/(+)	>1,100/(+)	>1,100/(+)	>1,100/(+)
Olho D'água Grande	< 3.0/(-)	240/(+)	290/(+)	460/(+)	43/(+)
Igreja Nova 1	< 3.0/(-)	240/(+)	3.0/ (+)	< 3.0/(-)	>1,100/(+)
Igreja Nova 2	< 3.0/(-)	< 3.0/(-)	>1,100/(+)	290/(+)	>1,100/(+)
Igreja Nova 3	3.6/(+)	6.1/(+)	1,100/(+)	>1,100/(+)	460/(+)
Porto Real do Colégio 1	15/(+)	7.4/(+)	>1,100/(+)	1,100/(+)	240/(+)
Porto Real do Colégio 2	15/(+)	11/(+)	>1,100/(+)	1,100/(+)	1,100/(+)

(+) = presence of *Escherichia coli*; (-) = absence of *Escherichia coli*

Table 4. Distribution of counting for coagulase-positive *Staphylococcus* in 58 samples of refrigerated raw milk, analyzed by colony forming unit parameter.

Region of the State of AL	**Counts of coagulase-positive *Staphylococcus* (CFU/mL)**				
	1st collection	***2nd collection***	***3rd collection***	***4th collection***	***5th collection***
Tanque D'arca	300	10	<10	<10	-
Mar Vermelho	100	<10	<10	20	-
Girau do Ponciano	30	40	<10	40	5,300
Traipu 1	30	20	<10	<10	<10
Traipu 2	40	30	<10	200	3,800
Traipu 3	<10	20	<10	40	6,900
Olho D'água Grande	1000	10	<10	<10	6,800
Igreja Nova 1	<10	2000	<10	<10	700
Igreja Nova 2	30	20	<10	300	4000
Igreja Nova 3	<10	1000	<10	30	9,500
Porto Real do Colégio 1	100	<10	<10	<10	1,200
Porto Real do Colégio 2	100	300	<10	<10	<10

conditions in the food and greater probability of the appearance of enteropathogens, suggesting low hygienic-sanitary conditions during the manipulation or improper storage procedures (Franco & Landgraf, 1996).

Other aspects related to the presence of Coliforms at 45 and *Escherichia coli* is the lack of hygiene at the place of milking, failures during the washing and sanitization of the utensils, bovine teats with feces, flaws in the handling of the milk during milking and use of water contaminated with fecal material (Brito, 2010). Non-virulent strains have the enteric tract of human and healthy animals as their habitat, while highly pathogenic strains are responsible for causing disease outbreaks in humans and animals with a significant mortality rate (ICMSF, 1998).

The microbiota of raw milk was studied in 300 properties of five Brazilian states (Minas Gerais, São Paulo, Paraná, Rio Grande do Sul and Pernambuco) and higher counts was found for aerobic mesophilic, psychrophilic bacteria, fecal coliforms, and *Escherichia coli*. However, the prevalence of the lactic acid bacteria (Beloti, 2015). Bacteria from the coliform group can multiply rapidly in milk, especially in the hottest months of the year, which ferment lactose producing lactic acid and other organic acids, which causes acidity in milk. The lack of hygiene in the handling of milk, especially the use of utensils that are not properly cleaned and the non-cooling or inadequate cooling of the milk are conditions favorable to the development of raw milk acidity (Brito et al., 2003).

3.1.2 Coagulase-positive Staphylococcus Counting

From the 58 analyzed samples, the coagulase-positive *Staphylococcus* counting presented values in the range of <10 CFU/mL to 6,900 CFU/mL and were detected in 33 (56.9%) raw milk samples (Table 4). The coagulase-positive *Staphylococcus* values keeping in the range between < 10 and 300 CFU/mL in the first, second, third and fourth collections. However, some rural associations (three rural associations) presented values up to 1000 CFU/mL in the first collection and values up to 2000 CFU/mL in the second collection for three rural associations. In this period, the rainfall is very intense. The risks of bovine mastitis caused by *Staphylococcus aureus* appear to be increased in the presence of elevated rainfall, possibly caused by relative humidity and temperature conditions favorable to their survival and proliferation (Zafalon et al., 2008). The results of the fifth collection were very discrepancy with the high counting of coagulase-positive Staphylococcus (values between 700 to 9,500 CFU/mL) for (08) eight rural associations. The climatic variations in very intense in the semiarid region of Brazilian northeast (Table 4) during this period (intermediate between the end of the summer season and the beginning of autumn).

There is no microbiological standard for the counting of coagulase-positive *Staphylococcus* in raw milk, but the high counting of this pathogen in the final whole milk product might contain enterotoxigenic strains that are thermo-resistant, indicating a potential danger to public health. This incidence of the coagulase-positive

Staphylococcus may be due to poor hygiene of teats during milking or even due to subclinical mastitis. The presence of mastitis characterizes a prominent development of microorganisms and somatic cells of milk being referred to *Staphylococcus aureus*, among other pathogens coming from diseased animals (ICMSF, 1998). Another possibility to be considered is another form of transmission along the manipulators, during the phases of contact with the product (Melo, 2010).

3.2 Bovine Thermal Comfort Index and Vegetation Index

The bovine thermal "discomfort" increases with the growth of the index, being able to reach levels of the effective health risk of the herd (INMET, 2015). The data of the Bovine Thermal Comfort Indices (BTCI) observed between July / 2014 and July / 2015 (INMET, 2015), through satellite data close to the Traipu and Penedo municipalities (Figure 2). The thermal sensation of the animal is influenced, among other factors, by temperature and relative humidity. In the case of cattle, empirical studies have shown that the Temperature and Humidity Index (THI) is a good indicator of the thermal comfort condition to which the animal is subjected. THI values above 72 (Attention level) impose discomfort on the cattle and can reach levels of effective herd health risk (INMET, 2015). The Attention, Alert and Danger ranges during the referred months, for presenting with (BTCI) above 72. The BTCI showed Alert and Danger levels during the summer and autumn (November 2014 and May 2015) for the Traipu and Penedo municipalities.

The vegetation index presented values between 0.75 and 0.39 during the months between the first and fifth collection (Figure 2). The low vegetation index (≤ 1.00) means that there is a lack of pasture in good quality and lack of nutrients necessary to the herd. The semiarid area has presented low vegetation index during all-weather seasons and has contributed to high contamination for coliforms at 45 ºC, *Escherichia coli*, and coagulase-positive *Staphylococcus* in the raw milk produced by the small rural associations.

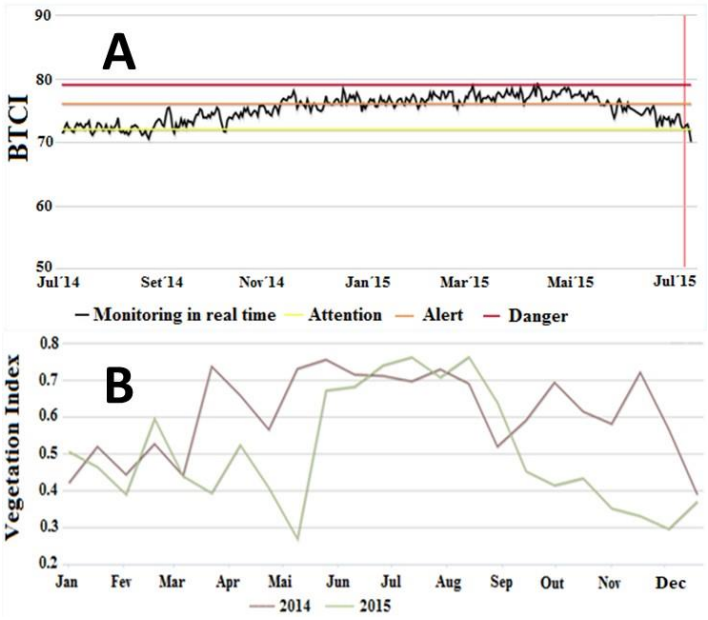

Figure 2. Timeline graphic for Bovine Thermal Comfort Index (A) in the Traipu and Penedo area during the period of July/2014 and July/2015 and Vegetation Index (B) in the same area during the years of 2014 and 2015.

The caloric stress in dairy cattle, especially in tropical regions, is an important source of economic loss of

livestock promoting an adverse effect on milk production, meat production, production physiology, reproduction, calf mortality and udder health, contributing with the highest number of coliforms in the beds of the animals (Ricci et al., 2013).

The presence of Coliforms at 45 ºC and *Escherichia coli* were evidenced in the months of November (third collection), January (fourth collection) and April (fifth collection) in relation to the average temperature of the regions (26 ºC). The maximum temperature (27 ºC) and the bovine thermal discomfort in the months between November (third collection) and April (fifth collection) were also in parallel with the microbiological data in evidence (3 of 5 collections, 60%). Citadin et al. (2009) analysed samples of raw milk in 31 dairy farms in the State of Paraná-Brazil, directly from expansion tanks or brass tanks, during the summer months and showed that 58.06% of the properties had Coliforms at 45 ºC with a minimum value of 4.00 x 10^{o} CFU/mL and a maximum of 1.10 x 10^{4} CFU/mL. In scientific literature, the rate of infection by environmental pathogens showed increased presence in summer and coincided with the largest number of coliforms in animal beds (Ricci, 2013).

In the survey between January 2008 and April 2009, the authors found that during the hottest months of the studied period (November to April), there was the highest incidence of condemnation for acid milk, with the highest amount of milk sentenced in the month of February of the year 2009. The count of mesophilic bacteria is commonly used to indicate the sanitary quality of food, and all foodborne pathogens are mesophilic (Franco & Langraf, 1996). Another scientific study point out there was a higher number of milk condemnations with high acidity, due to the influence that high temperatures exert on the growth of mesophilic bacteria, which find optimal conditions for their metabolism in tropical climate countries, especially considering the warm months of the year (Fava & Pinto, 2010).

In this study, coagulase-positive *Staphylococcus* also presented a congruence between the positive results in the samples and the climatological variable studied. A significant coagulase-positive *Staphylococcus* count was observed in relation to the maximum temperature of the regions (27 ºC) and bovine thermal discomfort at the time from the third, fourth and fifth collections characterized by the intermediate between the end of the summer season and the beginning of autumn. Other relevance studies about the relation between raw milk microbiota and the climate variation in semiarid areas from Brazil (southeast and northeast from Brazil) have been reported in scientific literature (Catão & Ceballos, 2001; Mendonça et al., 2001; Mendes et al., 2010; Da Silva et al., 2008; Silva et al., 2010; Tebaldi et al., 2008).

4. Conclusion

The presence of high counting of coliform at 45 ºC, *Escherichia coli*, and coagulase-positive *Staphylococcus* during summer end and the autumn beginning should be considered. Refrigerated raw milk does not provide safety to the direct consumption without pasteurization process because of the immaturity in the production process as regards the various aspects that involve animal health, environmental interferences, the handling of cattle, the production environment, as well weather seasons from the semiarid area from northeast of Brazil.

The quality of raw milk refrigerated on the studied regions deserve special attention as raw milk material, especially for the relevance of the microbiota that is part of the natural environment of the product, in order to overcome barriers and preventive attitudes that try to prevent the survival of pathogenic microorganisms in industrial processing.

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6. Declaration of Interest

There is no conflict of interest among the authors regarding the submission of this research paper.

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