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Occurrence of unstable non-acid milk in dairy farms in northern Tocantins state, Brazil

Ocorrência de leite instável não ácido (LINA) em propriedades leiteiras no norte do Tocantins

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ABSTRACT: The diagnosis of unstable non-acid milk (UNAM) in Brazil is overlooked due to the lack of information about this problem available to dairy farmers, mainly in the North region of the country. This report describes the occurrence of UNAM in three dairy farms of family farmers in northern Tocantins state, Brazil, who use animal feed based on *Urochloa brizantha* without mineral supplementation. The animals had been subjected to a poor feeding practice, with low quantity and quality, due to the dry period in the region and inappropriate nutritional management. Fourteen animals that presented alterations in milk characteristics were subjected to 72% alizarol test, California mastitis test, strip cup test, somatic cell count (SCC), boil test, physical-chemical and microbiological analyses, and search for undesirable substances. The time of lactation, udder health, and serum glucose level of animals, and soil and forage quality were also evaluated. The results showed 42% animals diagnosed with mastitis, 21% of them with clinical and 21% with subclinical mastitis; and 78% of animals with samples positive for the alizarol and boil tests, and Dornic acidity between 14° and 18°, characterizing positive cases for UNAM. Only one animal presented SCC above the limit established by the Brazilian legislation. The animals presented undamaged teats and no alteration in glycemia. Changes in nutritional management were recommended and done and the alizarol tests were redone after 14 days, showing all negative results for UNAM. The conclusion is that incorrect nutritional management is the main cause of UNAN in the studied cases.

KEYWORDS: Alizarol, Dornic, incidence, acidity, instability.

RESUMO: O diagnóstico do leite instável não ácido (LINA) no Brasil ainda é negligenciado devido à falta de informação dos produtores acerca do problema, principalmente na região norte do país. Este relato descreve a ocorrência de leite instável não ácido (LINA) em três propriedades de agricultura familiar localizadas no norte do Tocantins cuja alimentação dos animais era à base de *Urochloa brizantha* e sem suplementação mineral. Os animais passavam por restrição alimentar, com quantidade e qualidade prejudicadas em função do período de seca e de um manejo nutricional inadequado. Foi realizado teste do Alizarol 70%, California Mastitis Tests, Teste de Caneca Telada, CCS, teste da fervura, além de análises físico-químicas, microbiológicas e pesquisa de substâncias indesejáveis em 14 animais que apresentaram alterações em seu leite. Também foram avaliados o tempo de lactação, a saúde do úbere e os níveis de glicose sérica dos animais, além da qualidade do solo e das forrageiras oferecidas. 42% dos animais foram diagnosticados com mastite, destes, 21% clínica e 21% subclínica. 78% dos animais obtiveram amostra com resultado positivo no teste do alizarol e no teste da fervura e acidez Dornic entre 14° e 18, caracterizando casos positivos para LINA. Apenas um animal apresentou CCS acima do preconizado pela legislação. Os animais apresentaram tetos íntegros e não alteração da glicemia. Foram sugeridas mudanças no manejo nutricional, e após 14 dias foram refeitos os testes do Alizarol com todos resultados negativos para LINA. Concluiu-se que os casos estudados têm como principal causa o manejo nutricional incorreto.

PALAVRAS-CHAVE: Alizarol, Dornic, incidência, acidez, instabilidade.

INTRODUCTION

Brazil is the third largest milk producing country (FAO, 2019); the growing of the dairy sector in Brazil has been maintained by the high consumption per capita and its competitiveness in the international market (CARVALHO; ROCHA, 2020), which requires the supplying of high-quality products (WINCK et al., 2011).

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Despite the economic importance of this activity for the country, problems related to milk quality are faced by dairy industries, one of them is the unstable non-acid milk (UNAM), which is characterizing by the loss of casein stability in the milk, assessed by the alcohol test, without presenting increases in acidity (OLIVEIRA et al., 2011). This alteration has multifactorial causes, mainly connected to poor or imbalanced feed, long lactation time, thermal stress, and genetic factors (AZAMBUJA, 2018) (ZANELA; RIBEIRO, 2018).

The Normative Instruction 77 of 2018 of the Brazilian Ministry of Agriculture, Livestock, and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento, 2018b) establishes the alcohol and titratable acidity tests as the criteria for raw milk quality evaluations. UNAM is defined as the milk that present pellet formation in the alcohol test, titratable acidity below 18º Dornic, and formation of lumps in the boil test (ZANELA; RIBEIRO, 2018).

The processing of UNAM is not carried out by industries to avoid compromising the functioning and cleaning of equipment during the milk pasteurization (VOGES et al., 2018). In addition, the composition of this product presents alterations when compared to regular milk, with lower lactose and non-fat solid contents, which results in low yield of dairy derivatives (ZANELA et al., 2011). When UNAM is not correctly identified, it can be mistakenly interpreted as acid milk, penalizing farmers without them knowing the real problem in the herd (ZANELA; RIBEIRO, 2018).

Alterations in milk stability has been found in several states of Brazil, including Rio Grande do Sul (ZANELA et al., 2011), Rio de Janeiro (DONATELE; VIEIRA; FOLLY, 2003), São Paulo (BOTARO et al., 2009) (OLIVEIRA et al., 2011), Santa Catarina (WERNCKE, 2012), Paraná (BLASQUES et al., 2011) (LAZARROTO; DRUNKLER, 2012), and Pernambuco (PACHECO, 2011). However, as far as is known to date, no case has been reported for the state of Tocantins or to other states of the North region of country.

The present work reports the first case of occurrence of UNAM in crossbred bovine animals raised under extensive system in northern Tocantins state, Brazil, where this disease was diagnosed in three dairy farms.

REPORT OF CASE

The study was conducted in three dairy farms in northern state of Tocantins, Brazil, which had their products rejected by the quality control of industries: Farm 1 had 27 cows (Girolando and Caracu-Gir) in lactation and mean daily milk production of 212 liters; Farm 2 had 21 cows (Girolando) in lactation and mean daily milk production of 158 liters; and Farm 3 had 23 cows (Girolando and Swiss Brown) in lactation and mean daily milk production of 156 liters.

The production system of the dairy farms was extensive and characteristic of family farmers, with herds fed based on grasses (*Urochloa brizantha*) and water ad libitum. None of the dairy farms used supplemental concentrate or roughage feed. The cases occurred in September, final third of the dry period in the region, where the climate reaches mean maximum temperatures of 36 °C (34 to 38 °C), mean relative humidity of 55% (42% to 71%), and mean rainfall depths of 29 mm (0 mm to 114 mm). The dairy farms did not have any alternative method to reduce thermal stress of the animals, such as shading, ventilation, or sprinklers.

The milk of all cows was subjected to strip cup test, California mastitis test (CMT), and 72% alizarol test after refrigeration at 4 °C for four hours. Seventy-one animals were evaluated, 14 of them showed alterations in the results: 3 positives for the strip cup test, indicating clinical mastitis; 3 positives for the CMT, indicating subclinical mastitis; and 13 positives for the 72% alizarol test (TABLE 1). Therefore, 14 milk samples of positive animals were sent to the laboratory for microbiological and physical-chemical analysis (Dornic acidity, relative density, cryoscopy, fat, and non-fat dry extract), electronic count of somatic cells, boil test, and search for chlorides, alkaline, peroxides, blood, urine, and starch. The body condition score, lactation stage, udder integrity, and serum glucose levels of the animals, and the soil and forage quality were also evaluated. The positive and negative results of alizarol test are exemplified in Figure 1.

The results of total bacteria count of all samples analyzed were consistent with the values established by the Normative Instruction 76 (NI 76) (BRAZIL, 2018); only one animal (7) presented discrepant coliform value (45°) (TABLE 2). Regarding the physical-chemical parameters, 9 animals presented milk alterations in cryoscopy, 2 presented high acidity, and 7 presented non-fat solid contents below the minimum required by the NI 76. (TABLE 3). Only one animal of Farm 1 presented milk with SCC >500.000 CS ml^{-1}, which is above the limit established by the NI 76 (TABLE 3), 13 samples presented instability in the boil test (TABLE 3), and all samples presented negative results for the presence of alkaline and peroxide compounds, blood, urine, and starch. Eleven out of the 14 cows evaluated were positive for UNAM (TABLE 3), corresponding to 18% of the herd of Farm 1, 14% of the herd of Farm 2, and 13% of the herd of Farm 3.

The selected animals presented body condition scores between 2 and 3 and clinical examinations showed that their udders were undamaged and preserved. The serum glucose levels were also not altered, with values between 45 to 50 mg dL^{-1}. The soil and forage quality were evaluated in samples sent to the laboratory for analyses of pH, organic matter, phosphorus, potassium, calcium, magnesium contents, and determination of base saturation and cation exchange capacity. The results showed presence of low fertility soils, with acid pH (approximately 4.5), and forages with low nutritional value and low base saturations (16% to 28%).

After the identification of UNAM cases, the inclusion of 15 kg of sugarcane with addition of 0.5% to 1% of urea and protein salt in the diet of the cows was recommended and done

by the farmers, to reduce nutritional deficit and incidence of the problem. Fourteen days after this nutritional supplementation was implemented, the 72% alizarol tests were redone and all results were negative for UNAM.

DISCUSSION

This is of first report of UNAM in the state of Tocantins and in the North region of Brazil. It was diagnosed in crossbred bovine animals in three dairy farms that use extensive management system.

The correct diagnosis of UNAM requires to discard the possibility of acid milk through routine tests, including 72% alizarol test, Dornic acidity analysis, and stability test (boil test) in laboratory (ZANELA; RIBEIRO, 2018). Thus, unnecessary discard of milk can be avoided, since the use of tests of instability to alcohol does not hinder the manufacturing of products and does not pose a risk to health public (BRAZIL; NICOLAU; SILVA, 2015). The diagnosis of clinical and subclinical mastitis is also essential to elucidate the causes of the problem, since the disease

Table 1. Results of the California mastitis test, strip cup test, and 72% alizarol of 14 animals that presented changes in the three dairy farms evaluated.

Farm	Animal	Race	Months of Lactation	CMT	Strip Cup Test	Alizarol Test
Farm 1	1	Girolando	4	-	´´+``	+
	2	Girolando	4	-	-	+
	3	Girolando	4	-	-	+
	4	Caracu-Gir	9	-	-	+
	5	Girolando	9	-	´´+``	+
	6	Girolando	8	-	´´+``	+
Farm 2	7	Girolando	5	-	-	+
	8	Girolando	5	-	-	+
	9	Girolando	5	-	-	+
	10	Girolando	5	´´++``*	-	+
	11	Girolando	7	´´+``	-	-
Farm 3	12	Brown Swiss	5	-	-	+
	13	Girolando	3	-	-	+
	14	Girolando	2	´´+``	-	+

* = 0 to 200 thousand SC ml^{-1} or negative; + = 400 thousand to 1.5 million SC ml^{-1} or weakly positive; ++ = 800 thousand to 5 million SC ml^{-1} or positive; +++ = above 5 million SC ml^{-1} or strongly positive.

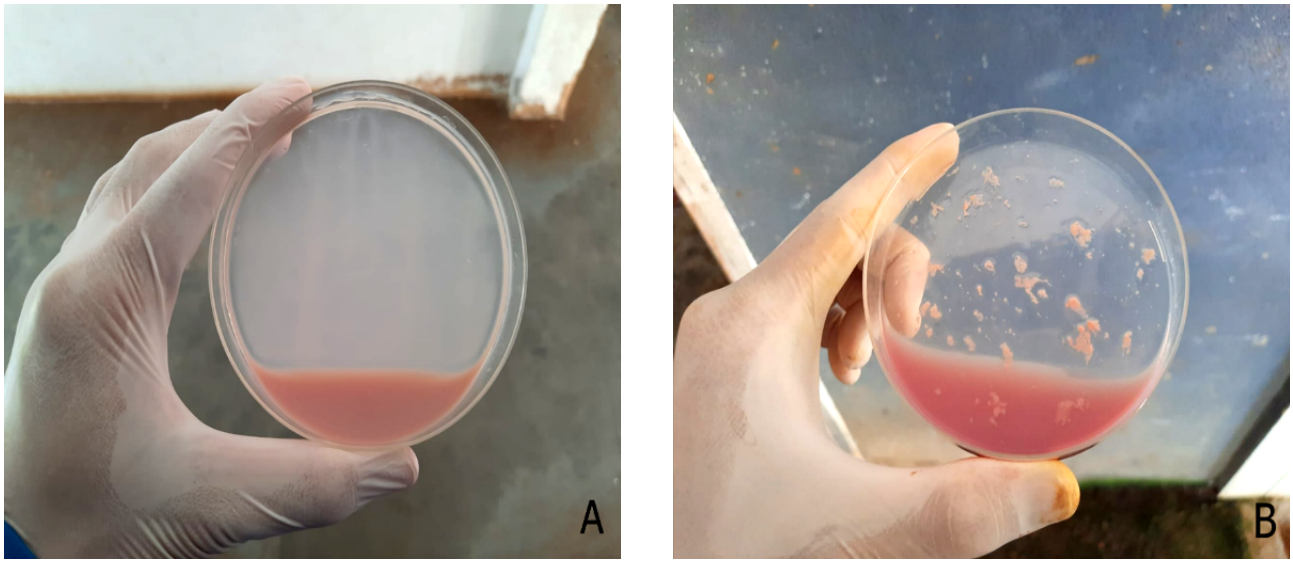

Figure 1. Milk samples with negative (A) and positive (B) results to the alizarol test.

changes the vascular permeability of secretor cells of the breast gland, affecting ionic concentrations and milk stability (PEREIRA et al., 2019).

Despite the production systems of the dairy farms were not technological, all evaluated samples were consistent with the Brazilian legislation (NI 76) regarding microbiological

Table 2. Microbiological analyses of milk samples of 14 animals that presented alterations in milk characteristics in the three dairy farms evaluated.

Farm	Animal	Total Bacterial Count (UFC/ml)	Coliforms 35° (MPN ml^{-1})	Coliforms 45° (MPN ml^{-1})	Yeasts (CFU ml^{-1})
Farm 1	1	<1x10⁴	<0.3	<0.3	74
	2	<1x10⁴	<0.3	<0.3	<74
	3	<1x10⁴	<0.3	<0.3	<74
	4	<1x10⁴	<0.3	<0.3	78
	5	<1x10⁴	<0.3	<0.3	78
	6	<1x10⁴	<0.3	<0.3	<74
Farm 2	7	<1x10⁴	<0.3	43	<74
	8	<1x10⁴	<0.3	<0.3	78
	9	<1x10⁴	<0.3	<0.3	<74
	10	<1x10⁴	<0.3	<0.3	78
	11	<1x10⁴	<0.3	<0.3	<74
Farm 3	12	<1x10⁴	<0.3	<0.3	<74
	13	<1x10⁴	<0.3	<0.3	<74
	14	<1x10⁴	<0.3	<0.3	<74

MPN = most probable number; CFU = Colony Forming Units

Table 3. Physical-chemical analyses and Somatic Cell Count of 14 animals that presented alteration in the milk in the three dairy farms evaluated.

Farm	Animal	Cryoscopy (°H)	Acidity (°D)	Density (g ml^{-1})	Fat (%)	NDE (%)	SCC (Cell mL^{-1})	Boil Test	UNAM
Farm 1	1	0.528	19	1.029	3.2	8.24	79,000	+	-
	2	0.533	15	1.031	3.3	8.82	530,000	+	+
	3	0.512	16	1.029	3.8	8.17	69,000	+	+
	4	0.528	18	1.029	3.2	8.24	98,000	+	+
	5	0.531	17	1.030	3.3	8.82	80,000	+	+
	6	0.512	16	1.031	3.8	8.17	95,000	+	+
Farm 2	7	0.528	15	1.028	3.9	8.13	147,000	+	+
	8	0.535	17	1.031	3.5	8.86	79,000	+	+
	9	0.526	20	1.031	4.5	8.80	69,000	+	-
	10	0.528	17	1.031	3.4	8.74	160,000	+	+
	11	0.528	17	1.030	3.6	8.75	85,000	-	-
Farm 3	12	0.512	16	1.029	3.8	8.17	69,000	+	+
	13	0.528	18	1.029	3.2	8.24	98,000	+	+
	14	0.531	17	1.030	3.3	8.82	80,000	+	+

NDE non-fat dry extract; SCC = somatic cell count; UNAM = unstable non-acid milk

quality and absence of undesirable substances. Good hygiene practices during milking and correct cooling of milk directly affect the maintenance of these parameters, as shown by Mesquita et al. (2018).

Regarding the results of the physical-chemical analyses of the UNAM, the means found for Dornic acidity, fat, and non-fat dry extract were similar those described by Lopes (2008), who conducted a study on UNAM in small dairy farms in the interior of São Paulo state, Brazil, in the same period of the year. Changes in SCC was evident in one animal (2) of Farm 1, which may have affected the positive result for UNAM according to Brazil; Nicolau; Silva (2015), since increases in this parameter increase the contents of plasmin and other proteases of the blood or produced by defense cells, which hydrolyze casein leaving it unstable to heat (CORASSIM; ROSIM; KOBASHIGAWA, 2013).

The animals had been subjected to a poor feeding practice, with low quantity and quality, due to the dry period in the region and inappropriate nutritional management. Although the causes of occurrence of UNAM is still not fully understood, studies have correlated it to nutritional deficits and lack of nutritional supplementation in this period (OLIVEIRA et al., 2020). These factors can increase the apoptosis of the breast epithelial cells, modify the blood composition and pH and, consequently, alter milk composition and its capacity to resist to alcoholic dehydration (MARQUES et al., 2010). Thermal stress should also be considered, since it decreases feed ingestion and increases respiratory frequency and peripheral vasodilation of animals, decreasing nutrient availability for the synthesis of milk (SCHMIDT, 2014). Abreu et al. (2011) found decrease in milk production from 20.3 to 12.7 liters and decreases in milk stability, assessed by the alcohol test, from 76.41% to 70.83% after the animals were maintained in a non-shaded environment for five consecutive days

The lactation stage can also affect milk stability. In the initial stage of the lactation, this instability can be correlated to alterations in concentration of proteins and divalent cations, cation to anion ratio, and salt balance (ROSA et al., 2017). Cows at advanced lactational stages can also present low milk stability, even when well fed and presenting no metabolic diseases (MARQUES et al., 2010).

Correct soil and forage managements are essential to minimize the occurrences of this problem. Production systems that use more roughage supplementation and grazing in pastures with low neutral detergent fiber contents for the diet of animals, present lower occurrence of milk with instability (VOGES et al., 2018). Feed with quality grasses and supplementation of vitamins, mineral, limestone, and salt results higher casein, lactose, and fat contents in the milk produced and reduce cases of UNAM in the herd (GABBI et al., 2013).

The conclusion is that the UNAM cases found in the dairy farms studied were due to incorrect nutritional management, which is correlated to low-fertility soils and low-quality pastures, effects of thermal stress, and lactation stage of the animals.

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