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Multifactorial analysis of unstable non-acid milk occurrence in dairy production

Análise multifatorial da ocorrência de leite instável não ácido em propriedades leiteiras

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ABSTRACT
The aim was to analyze the Unstable Non-Acid Milk (UNAM) phenomenon through the association of zootechnical characteristics of the farms relating them to their occurrence. The data collection was carried out in 12 dairy farms which received a technical visit to learn the production system: handling, feeding, mineralization and milking procedures. The milk samples were analyzed for fat, protein, lactose, solids not fat, standard plate count, somatic cell count, pH, titratable acidity and ethanol test stability. Milk data were submitted to analysis of variance; association between zootechnical characteristics and UNAM were determined by logistic regression; and multivariate analysis of principal components was carried out. The occurrence of UNAM was verified in 25% of the evaluated farms. In UNAM herds 27% to 33% of the cows were classified as UNAM. The animals with UNAM milk was different between farms classified as Stable or UNAM, being 12% and 30% of cows, respectively. The detection of UNAM was possible when in the herd there are at least 23% of the animals producing UNAM milk. The three principal components identified covered the main areas of influence on the occurrence of UNAM: Quality and interaction with part of the solids; Production and relationship with lactose; Nutrition and correlations with part of the components. The classification of milk in the expansion tank as UNAM can only be identified when more than 23% of the animals were in this condition. The multivariate analysis identified that the nutritional balance of each cow was determining factor for UNAM presence.

Keywords: cow, nutrition, production system.

RESUMO
Objetivou-se analisar o fenômeno do Leite Instável Não-Ácido (LINA) por meio da associação das características zootécnicas das fazendas, relacionando-as com sua ocorrência. A coleta de dados foi realizada em 12 propriedades leiteiras que receberam visita técnica para conhecer o sistema de produção: manejo, alimentação, mineralização e ordenha. As amostras de leite foram analisadas quanto a gordura, proteína, lactose, extrato seco desengordurado, contagem padrão em placa, contagem de células somáticas, pH, acidez titulável e estabilidade à prova do álcool. Os dados do leite foram submetidos à análise de variância; a associação entre características zootécnicas e LINA foi determinada por regressão logística; e foi realizada análise multivariada de componentes principais. A ocorrência de LINA foi verificada em 25% das fazendas avaliadas. Nos rebanhos LINA, 27% a 33% das vacas foram classificadas como LINA. Os animais com leite LINA diferiram entre as fazendas classificadas como Estável ou LINA, sendo 12% e 30% vacas, respectivamente. A detecção de LINA foi possível quando no rebanho havia pelo menos 23% dos animais com leite LINA. Os três principais componentes identificados abrangeram as principais áreas de influência na ocorrência de LINA:

Qualidade e interação com parte dos sólidos; Produção e relação com a lactose; Nutrição e correlações com parte dos componentes. A classificação do leite do tanque de expansão como LINA só pode ser identificada quando mais de 23% dos animais estavam nessa condição. A análise multivariada identificou que o balanço nutricional de cada vaca foi fator determinante para a presença de LINA.

Palavras-chave: nutrição, sistema de produção, vaca.

1 INTRODUCTION

Nowadays, protein is the most valuable milk component worldwide, and the preference for this component over fat milk has become an important trend in dairy production, especially considering how conscious milk' consumers are about feed nutritional value and its impacts over human health (ROMA JUNIOR et al., 2016). Such consciousness could be perceived by increasing interest in cheese and consumers' stimulation to reduced fat consumption. In addition, the content and quality of milk protein are other important aspects, once it directly influences milk derivatives' industrial production, mostly cheese (GOULART et al., 2019).

The protein quality in dairy production has relation to milk' thermal stability, especially its ability to resist thermal processing (BARBOSA et al., 2017; MACHADO et al., 2017). In this process, it is possible to happen certain changes in the protein structure, such as protein micelles (MANSKE et al., 2020).

The prediction of thermal stability in milk protein using a quick estimation process through milk ethanol stability is still widely used in Brazilian dairy industries, as a way to obtain a high-quality raw milk (RATHNAYAKE et al., 2016), due to its lower bacterial contamination (FAGNANI et al., 2016a).

When thermal instability of milk' protein fractions not related to milk acidity caused by microbial origins, it occurs the unstable non-acid milk (UNAM), which is directly related to the quality of milk protein (FAGNANI et al., 2016b). The UNAM presents pH and normal titratable acidity, lower bacterial and somatic cell count, also visible clotting under alcohol test (GOULART et al., 2019).

The occurrence of this event is still under study owing to the complexity of the factors involved in the thermal stability of milk and its random occurrence, imposing the challenge of generating knowledge in search of control and / or preventive measures (RATHNAYAKE et al., 2016; VOGES et al., 2018; GOULART et al., 2019; MANSKE et al., 2020). In view of the aspects approach in relation to the quality of milk protein, the objective of the present study was to analyze the UNAM phenomenon through the association of zootechnical characteristics of the properties, relating them to their occurrence.

2 MATERIAL AND METHODS

The data collection was carried out in 12 dairy farms, 4 farms in the region of Poços de Caldas, MG State, and 8 farms in the region of Guaratinguetá, SP State. Each farm received a technical visit to learn about the production system: handling, feeding, mineralization and milking procedures. For that, the handling of the animals was monitored before, during and after the complete milking.

Through an interview with the technician responsible for the farm, the date of the last calve was verified, for later calculation of the lactation stage. All animals were evaluated by the same technician for body condition score through muscle coverage, fat deposition on the tail, side, scapula and lumbar region, with scores ranging from 1 to 5, with an interval from 0.5 points, as described by Ruas et al. (2000).

Each animal had it is daily milk production measured and, during the morning milking, a sample of 50 mL of milk was collected, in Bronopol® conserving. For each property visited, at the end of the day's milking, two milk samples (50 mL) were collected directly from the expansion tank. The Bronopol® conserving was used in one sample and the Azidiol® conserving in the other.

The milk samples preserved with Bronopol® were analyzed for fat content, protein, lactose, solids not fat, standard plate count (SPC), pH, titratable acidity and stability in the ethanol test. For milk samples preserved with Azidiol®, microbiological evaluation was performed using the SPC.

The individual milk samples were analyzed for fat, protein, lactose, solids not fat, somatic cell count (SCC), pH, titratable acidity and stability in the ethanol test. The analysis of milk composition in terms of protein, fat, lactose and solids not fat was performed electronically by infrared absorption in the Bentley 2000 equipment (BENTLEY INSTRUMENTS, 1995a).

The SPC and SCC were analyzed using flow cytometry methodology, using the IBC Bactocount® and Somacount® equipment, respectively, at the Lactation Physiology

Laboratory of Milk Clinic / ESALQ / USP (BENTLEY INSTRUMENTS, 1995b; (BENTLEY INSTRUMENTS, 2004).

The pH was measured immediately after receiving the samples, using a digital potentiometer. Quantification of titratable acidity was performed by titration with sodium hydroxide (0.1N), using the alcoholic phenolphthalein solution (1%) as an indicator, according to the National Animal Reference Laboratory (BRASIL, 1981).

In addition, the stability of the milk in the alcohol test was determined, where 2 ml of milk and 2 ml of ethanol were placed in Petri dishes, homogenizing them. The results were scored on a scale of 1 to 5 as described by Balbinotti et al. (2002) being: 1 - stable milk with no precipitation; 2 - unstable milk with light precipitation (lumps similar to sand); 3 - unstable milk with medium precipitation (thicker lumps evenly distributed throughout the plate); 4 - unstable milk with intense precipitation (formation of larger clumps located in some points of the plate) and 5 - unstable milk with very intense precipitation (formation of a clot similar to a grid).

To assess thermal stability, a standard curve was constructed based on three alcohol concentration values, being: 72% as required by Brazilian law, 78% which was the standard used by the dairy participating in the project and 75% adopted as the chosen intermediate value for building the standard curve. Through the standard curve constructed with the data of the samples in the three alcohol concentrations, the stability value was estimated according to the alcohol concentration, that is, the limit point of alcohol concentration for which the sample reached the coagulation value “4” the scale suggested by Balbinotti et al. (2002).

The samples were classified as UNAM (CENLAC, 2004) only when they presented positive results in the three tests: visible coagulation in the alcohol test, titratable acidity less than 18°D and pH greater than 6.6. Samples that did not show visible coagulation in the 78% alcohol test were classified as Stable. Samples that showed visible coagulation in the alcohol test 78%, but with a pH below 6.6, as well as samples with SPC above 1,000,000 cfu/mL and SCC above 1,000,000 cells/mL were classified as Unstable.

The data that did not present a normal distribution were categorized according to the assessed item, as described below. The lactation stage was divided into 5 classes, namely: 1) 0 to 45 days; 2) 46 to 100 days; 3) 101 to 200 days; 4) 201 to 300 days; 5) greater than 301 days of lactation. The body condition score was divided into 5 classes: 1) score less than 1; 2) score between 1.5 and 2; 3) score between 2.5 and 3; 4) score between 3.5 and 4; 5) score greater than 4.5. Milk production in 3 classes: 1) 0 to 10 L/day; 2) 10.1 to 20 L/day; 3) greater than 20.1 L/day. The fat-protein ratio in 3 classes: 1) less than 1; 2) between 1.1 and 1.5; 3) above 1.5. And for the classification of urea nitrogen in milk 3 classes: 1) less than 8 mg / dL; 2) between 8.1 and 25 mg/dL; 3) above 25.1 mg/dL.

To approximate the normal distribution of the data, the SCC values were converted into somatic cell scores (SCS) (SHOOK et al., 2017), following the equation: SCS = log2 (SCC/100) + 3. And the data of SPC were transformed using the base 10 logarithmic function.

The data on stability, titratable acidity, pH, fat, protein, lactose, solids not fat, SCC, SPC were submitted to analysis of variance using the SAS® GLM procedure (SAS Inst. Inc., Cary, NC).

To determine the association between zootechnical characteristics, the farm and the occurrence of UNAM, logistic regression analyzes were performed, using the LOGISTIC procedure of SAS®.

The multivariate technique of principal components was also applied to the data through the BIPLOT analysis of SAS®, considering all the data collected with the exception of data used to classify the samples, in order to avoid influencing the results. The excluded parameters were SCC, pH, titratable acidity and alcohol stability.

3 RESULTS AND DISCUSSION

In none of the 12 farms evaluated through the milk samples obtained in the cooling tank was classified as Unstable, therefore the data presented and the considerations will only be for the occurrence of farms classified as Stable and UNAM.

The occurrence of UNAM was verified in 25% of the evaluated farms (Table 1) and the volume of milk in this condition varied from 26% to 30% of the total production of each farm, therefore it is an important volume of milk to be discarded and that will occur on all farms for some animals. In UNAM herds, 27% to 33% of the cows evaluated were classified as UNAM. Thus, it can be said that this phenomenon is related to animals, not reflecting the general conditions in which the milk was produced.

Table 1. Farm classification, case quantification and volume of milk identified with UNAM in the participating farms.

Farm classification	Animals in lactation	Sample with UNAM		Total Volume	Volume of UNAM	
	n	n	%	L/day	L/day	%
1 (Stable)	53	4	8	1,297.1	92.8	7
2 (Stable)	17	3	18	167.5	23.3	14
3 (Stable)	36	5	14	336.7	36.9	11
4 (Stable)	43	6	14	808.5	115.0	14
5 (Stable)	19	3	16	246.1	32.7	13
6 (Stable)	23	2	9	297.5	33.8	11
7 (UNAM)	46	12	27	827.5	227.5	26
8 (Stable)	77	13	17	788.8	126.3	16
9 (Stable)	50	5	10	591.7	69.2	12
10 (Stable)	65	2	3	1,077.5	20.0	2
11 (UNAM)	33	11	33	443.8	135.0	30
12 (UNAM)	20	6	30	184.0	52.4	29
TOTAL	482	72	15	7,066.7	964.9	14

1. * Classification of samples collected in the expansion tank (Stable and UNAM).

Although the alcohol test is questionable as to its effectiveness in detecting acidic milk due to the need to have a rapid test (RATHNAYAKE et al., 2016) and to comply with Brazilian legislation (BRASIL, 2018), it is still widely used. On the other hand, the identification of thermal milk instability by the alcohol test (BARBOSA et al., 2017) could be used to direct the processing of milk and not just to discard it (GOULART et al., 2019). Thus, up to a quarter of the properties would not be penalized, if the other characteristics were within the acceptable range.

The occurrence of UNAM can be considered low due to the rigor of the test applied, alcohol at 78%, since the value of 76% has been suggested to identify better quality milk (MANSKE et al., 2020), even though higher values used. Another factor that can lead to lower incidence is the time of year, when the best nutritional conditions decrease the incidence of UNAM (MACHADO et al., 2017).

The number of animals that presented UNAM milk was different ($P<0.05$) between farms classified as Stable or UNAM, being 12% and 30% of cows, respectively. Thus, we can consider that the detection of UNAM is possible when in the herd there are at least 23% of the animals producing milk classified as UNAM.

In terms of the total volume of milk produced in each classification, a difference ($P<0.05$) was observed between the properties Stable and UNAM, being that 11% and 29% of the production were unstable, respectively. According to the minimum significant difference observed by the statistical test from 23% UNAM in the total volume of milk present in the expansion tank, it would already be possible for the entire production to be classified as UNAM.

Alcohol testing is an indirect way of trying to identify acidity of microbiological origin (FAGNANI et al., 2016a), but it is also influenced by other factors, especially those of feed origin that can alter the composition of milk, such as the salt balance, lactose content, urea nitrogen and ionic calcium (SOUZA et al., 2016).

There was no significant correlation (P <0.05) between the characteristics of the properties, such as race, management of feeding and milking the herds with the occurrence of UNAM (Table 2).

Table 2. Pearson's correlation coefficient and level of significance for each characteristic of the properties evaluated in relation to the occurrence of UNAM.

Characteristic	Correlation Coefficient	Significance
Breed	-0.174	0.588
Production system	-0.174	0.588
Pasture type	-0.516	0.103
Bulky type	0.559	0.073
Grass type	0.333	0.518
Origin of concentrate	-0.149	0.661
Mineral supplementation	-0.292	0.355
Milking system	-0.192	0.549

The occurrence of UNAM is a multi-factorial event being difficult to identify a single source of occurrence of this phenomenon (ROSA et al., 2017; SILVA et al., 2019; MANSKE et al., 2020), as shown in Table 2, where the factors were considered in isolation. The causes seem to be more related to the individual characteristics of the animals in the herds (TROCH et al., 2017), since there is no homogeneity of the lactating cows regarding the nutritional requirement, production level, age, heat sensitivity, pregnancy, health of the mammary gland, access to feed within the flock, influencing the nutritional status of each animal (MARTINS et al., 2015).

There was a difference (P<0.05) for the qualitative characteristics of the farms' milk between the Stable and UNAM for titratable acidity (Table 3), with higher values for the Stable units. This result confirms that UNAM's instability is not exclusively related to greater acidity (SOUZA et al., 2016; MACHADO et al., 2017) being yet another indicator that UNAM can be used for specific processing (FAGNANI et al., 2016a).

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Table 3. Comparison of milk composition according to the classification of the expansion tank samples of the 12 farms.

	Stable (n=9)	UNAM (n=3)
Degree of alcohol stability (%)	81[a]	77[b]
Titratable acidity (° D)	18.20[a]	15.60[b]
pH	6.63	6.65
Fat (%)	4.03	3.90
Protein (%)	3.19	3.14
Lactose (%)	4.46	4.44
Degreased dry extract (%)	8.52	8.43
Somatic cell count (1,000 cells/mL)	609	650
Standard plate count (1,000 cfu/mL)	36	20

Means followed by different letters on the same line differ (P<0.05) by F Test.

As expected, most of the milk components were not changed when the milk was classified as UNAM (SOUZA et al., 2016). Fagnani et al. (2016a) did not observe a direct relationship between bacterial count (up to 105 bac/mL) with UNAM in the same way that there was no direct relationship between SPC and pH and lactose (GOULART et al., 2019) as occurs in acid milk.

The individual evaluation of the animals was realized with 482 cows, distributed in the 12 farms, of which the milk was analyzed for composition and SCC (Table 4). Of these, 257 were classified as Stable, 72 as UNAM and 153 as Unstable.

Table 4. Comparison of milk composition according to individual classification of samples of the animals studied.

	Stable	UNAM	Instable
Degree of alcohol stability (%)	83[a]	74[b]	74[b]
Titratable Acidity (° D)	17.4[b]	15.9[c]	19.5[a]
pH	6.68[a]	6.67[a]	6.57[b]
Fat (%)	3.97	3.96	4.03
Protein (%)	3.21[ab]	3.16[b]	3.29[a]
Fat-Protein Ratio	1.24	1.26	1.21
Lactose (%)	4.48[a]	4.34[b]	4.35[b]
Degreased Dry Extract (%)	8.52	8.35	8.55
Urea Nitrogen in Milk (mg/dL)	16.76	15.96	15.61
Somatic Cell Count (1,000cells/mL)	348	280	268

Means followed by different letters on the same line differ (P<0.05) by Tukey test.

The samples of unstable milk were identified especially due to the higher titratable acidity, higher protein content compared to UNAM, lower pH than the others and lower lactose content compared to Stable. Thus, milk that is unstable due to contamination is characterized by acidity from bacterial growth (FAGNANI et al., 2016a), distinguishing it from UNAM as observed in the present study.

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For a more in-depth analysis of the occurrence of UNAM, logistic regression analyzes were performed, which were significant (P<0.05) for the categorized data of lactation stage and body condition score (Table 5).

Table 5. Odds Ratio values, 95% confidence interval and probability of occurrence estimated by the multivariate logistic regression method according to the categorized variables

Item	Estimate	Odds Ratio	Confidence Interval	P
Intercept	0.3011	0.755	0.508 – 1.123	0.7897
Milk production	-0.2809	1.385	1.078 – 1.780	0.1654
Lactation Stage	0.3257	1.069	0.615 – 1.857	0.0109
Urea Nitrogen in Milk	0.0664	0.993	0.646 – 1.525	0.8138
Fat-Protein Ratio	-0.00745	0.488	0.311 – 0.767	0.9729
Body Condition Score	-0.7170	0.755	0.508 – 1.123	0.0019

The lactation period has an effect on protein stability, focusing on the occurrence of UNAM. This can occur from the beginning of lactation to the peak of production, when there is greater nutritional demand, especially in primiparous cows (ROSA et al., 2017; TROCH et al., 2017) or due to the higher concentration of milk proteins in the advanced stages of lactation (SILVA et al., 2019), which implies greater formation of the β-lactoalbumin/κ-casein complex (MARTINS et al., 2015). According to the logistic regression analysis, there was no difference for milk production in relation to the occurrence of UNAM, which may be related to the greater supply of nutrients in balanced diet (VOGES et al., 2018).

The body condition score indicated that animals affected with milk instability were more likely to have a lower body condition, as evidenced by the negativity of the estimate (Table 5). Body condition can be indicative of nutritional or sanitary problems (ROSA et al., 2017; VOGES et al., 2018; SILVA et al., 2019) which directly affect milk quality.

The correlation analysis between the non-classificatory variables was performed considering only the milk quality components (Table 6).

Table 6. Correlation matrix of the analysis of main components for the study of the occurrence of Unstable Non-Acid Milk (UNAM).

	UNAM	Protein	SNF	Ash	Lactose	MUN	Fat
UNAM	1.0000	-0.0791	-0.1785	-0.1083	-0.1752	-0.0702	-0.0438
Protein		1.0000	0.7710	0.9827	-0.1153	-0.2642	0.3487
SNF			1.0000	0.8720	0.5435	-0.0696	0.2123
Ash				1.0000	0.0640	-0.2207	0.3268
Lactose					1.0000	0.2391	-0.1284
MUN						1.0000	0.0626
Fat							1.0000

SNF: solids not fat; MUN: milk urea nitrogen; UNAM: occurrence of Unstable Non-Acid Milk.

The same seven quality values were submitted to multivariate analysis to identify the number of main components, which was performed considering an accumulated eigenvalue above 75% (Table 7), which was obtained with three main components, in the amount of 79.04%.

Table 7. Description of the eigenvalues for the main components used in the analysis of the occurrence of Unstable Non-Acid Milk (UNAM).

Component	Eigenvalue	Difference	Proportion	Accumulated
1	2.982713	1.472086	0.4261	0.4261
2	1.510627	0.471000	0.2158	0.6419
3	1.039628	0.138430	0.1485	0.7904
4	0.901197	0.336608	0.1287	0.9192
5	0.564590	0.563344	0.0807	0.9998
6	0.001245	0.001245	0.0002	1.0000
7	0		0	1.0000

The three principal components (PC) identified covered the main areas of influence on the occurrence of UNAM: Quality (PC1) - and its interaction with part of the solids; Production (PC2) - and its relationship with lactose; Nutrition (PC3) - and it is correlations with part of the components (Table 8).

Table 8. Description of the principal components used to analyze the occurrence of Unstable Non-Acid Milk (UNAM).

	Principal Components (PC)		
Variable	PC1	PC2	PC3
Fat	0.237529	-0.175187	0.752056
Protein	0.545104	-0.211180	0.001195
Lactose	0.111239	0.712159	-0.220574
SNF	0.531989	0.277968	-0.140300
Ash	0.567375	-0.084089	-0.038202
MUN	-0.129601	0.466399	0.583642
UNAM	-0.111101	-0.340113	-0.154841

SNF: solids not fat; MUN: milk urea nitrogen; UNAM: occurrence of Unstable Non-Acid Milk.

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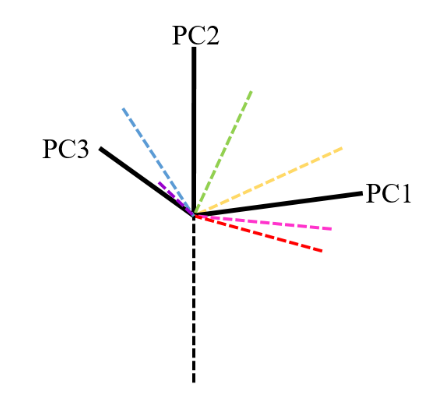
It is also possible to view the qualitative characteristics of the milk and the occurrence of UNAM in a graph (Figure 1), where it is possible to identify the variables that make up each PC.

Figure 1. Distribution of the studied variables according to the principal components (PC) identified.



Principal Components Analysis Biplot	
Variable	**Reference**
UNAM	
Protein	
Solids Not Fat	
Ash	
Lactose	
Fat	
Milk Urea Nitrogen	

The PC1 Quality, which includes milk protein, solids not fat (SNF) and ashes, is what most explains the occurrence of UNAM, concentrating 42.61% of the variation. Of the three variables, the protein content in milk is the most important, as it is included in the SNF and also because it is the fraction containing the casein micelles, whose stability is influenced by nutrition (MARTINS et al., 2015).

As accurate as animal feeding is, it is difficult to fully meet the individual requirements of cows (MARTINS et al., 2015), with a small part of the animals always producing UNAM, although temporarily (TROCH et al., 2017). The ash content that helps to compose PC1 reflects the importance of the mineral balance of the diet, which can imply changes in the balance of salts, accumulating ionic calcium in the milk and changing its pH (BARBOSA et al., 2017; VOGES et al., 2018).

The PC2 Production is only related to the lactose content and explains 25% of the factors related to UNAM, with the highest identified eigenvalue of 0.71. The passage of minerals into the milk can be unbalanced when there is a decrease in the lactose content, which occurs in cases of energy deficiency in the diet caused by the inefficient supply of glucose to the mammary gland, increasing the solubilization of ionic calcium and decreasing the pH of the milk. Thus, the stability of the casein micelles is compromised and UNAM may occur (MANSKE et al., 2020).

Therefore, the inverse relationship between the occurrence of UNAM and lactose content observed in the present study, evidencing that there was a glucose deficit in the mammary gland. In other words, many cases of the occurrence of this phenomenon may be closely related to the animals' energy deficiency.

PC3 Nutrition includes the variables fat and milk urea nitrogen, still as relevant factors for the occurrence of UNAM, even though it contributes less than 15%, reinforcing the importance of nutritional balance for milk production with greater thermal stability. It was found that fat (representing the energy fraction of the diet) and milk urea nitrogen (protein component of the diet) express the difficulty in achieving in practice complete efficiency in the process of meeting the total nutritional requirements of lactating animals (FICHER et al., 2012; SOUZA et al., 2016; ROSA et al., 2017; SILVA et al., 2019; MANSKE et al., 2020)

The occurrence of UNAM was observed in all herds. However, the classification of milk in the expansion tank in this condition can only be identified when more than 23% of the animals were in this condition.

The multivariate analysis considering the multiple factors involved in the UNAM phenomenon identified that the nutritional balance of each animal was the determining factor for the occurrence of UNAM. It was evident that the supply of energy, protein and minerals, as well as the balance between them, is essential for the production of milk with greater thermal stability.

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REFERENCES

BALBINOTTI, M.; MARQUES, L.T.; FISCHER, V.; RIBEIRO, M.E.R.; STUMPF, W.; RECKZIEGEL, F.J.; CARBONARI, C.; VARELA, M. Incidência do leite não ácido (LINA) na região Sul do Rio Grande do Sul. In: CONGRESSOM PAN AMERICANO DE QUALIDADE DO LEITE E CONTROLE DA MASTITE, 2. 2002, Ribeirão Preto, **Anais**... Ribeirão Preto: Instituto Fernando Costa, 2002.

BARBOSA, R.S.; VON HAUSEN, L.J.O.; FISCHER, V.; RIBEIRO, M.E.R.; STUMPF JUNIOR, W.; BONO, G.; BARROS, L. Efeito do período de lactação e estabilidade do leite sobre as características físico-químicas. **Revista Brasileira de Nutrição Animal**, v.11, n.1, p.14-18, 2017.

BENTLEY INSTRUMENTS. **Bentley 2000**: operator´s manual. Chaska, 1995a, 77p.

BENTLEY INSTRUMENTS. **Somacount 300**: operator´s manual. Chaska, 1995b, 12p.

BENTLEY INSTRUMENTS. **Bactocount 150**: operator´s manual. Chaska, 2004, 35p.

BRASIL. Ministério da Agricultura. Secretaria Nacional de Defesa Agropecuaria. Laboratorio Nacional de Referência Animal. **Métodos analíticos oficiais para controle de produtos de origem animal e seus ingredientes.** Brasil, v2, 1981.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 76, de 26 de novembro de 2018. Estabelece os Regulamentos Técnicos que fixam a identidade e as características de qualidade que devem apresentar o leite cru refrigerado, o leite pasteurizado e o leite pasteurizado tipo A, na forma desta Instrução Normativa e do Anexo Único. **Diário Oficial [da] República Federativa do Brasil**, Brasília, DF, 26 nov. Seção 1, p. 1, 2018.

CENLAC – Centro de ensayos para el control de la calidad de lácteos. **Manual de procedimentos técnicos operacionales.** Available in: <http://www.exopol.com/circulares/142.html>. Access in: 17 out 2015.

FAGNANI, R.; BATTAGLINI, A.P.P.; BELOTI, V.; ARAÚJO, J.P.A. Estabilidade do leite ao álcool ainda pode ser um indicador confiável? **Ciência Animal Brasileira**, v.17, n.3, p.386-394, 2016a. DOI: 10.1590/1089-6891v17i331848

FAGNANI, R.; BATTAGLINI, A.P.P.; BELOTI, V.; URBANO, A.; BRONZOL, J.C. Alcohol stability of milk from the perspective of X-ray diffractometry. **Food Biophysics**, v.11, p.198-205, 2016b. DOI 10.1007/s11483-016-9430-y

FISCHER, V.; RIBEIRO, M.E.R.; ZANELA, M.B.; MARQUES, L.T.; ABREU, A.S.; MACHADO, S.C.; FRUSCALSO, V.; BARBOSA, R.S.; STUMPF, M.T. Leite instável não ácido: um problema solucionável? Revista Brasileira de Saúde e Produção Animal, v.13, n.3, p.838-849, 2012.

GOULART, J.Q.; PICCININI, A.; PINTO, A.T. Caracterização do queijo fresco produzidos com leite de diferentes graus de estabilidade ao etanol. **PUBVET**, v.13, n.12, p.1-7, 2019. https://doi.org/10.31533/pubvet.v13n12a476.1-7

MACHADO, S.C.; FISCHER, V.; STUMPF, M.T.; STIVANIN, S.C.B. Seasonal variation, method of determination of bovine milk stability, and its relation with physical, chemical, and sanitary characteristics of raw milk. Revista Brasileira de Zootecnia, v.46, n.4, p.340-347, 2017. http://dx.doi.org/10.1590/S1806-92902017000400010

MANSKE, G.A.; DANIELI, B.; ZUFFO, G.R.; RIGO, E.; GOMES, F.J.; ZAMPAR, A.; SCHOGOR, A.L.B. Occurrence of Unstable non-acid milk (UNAM) on commercial farms in the extreme west of Santa Catarina. **Research, Society and Development**, v.9, n.7, p.1-18, e715974654, 2020. DOI: http://dx.doi.org/10.33448/rsd-v9i7.4654

MARTINS, C.M.M.R.; ARCARI, M.A.; WELTER, K.C.; NETTO, A.S.; OLIVEIRA, C.A.F.; SANTOS, M.V. Effect of dietary cation-anion difference on performance of lactating dairy cows and stability of milk proteins. Journal of Dairy Science, v.98, p.2650-2661, 2015. http://dx.doi.org/ 10.3168/jds.2014-8926

RATHNAYAKE, R.; MANGALIKA, U.; ADIKARI, A.; NAYANANJALIE, W. Changes in Compositional and Keeping Quality Parameters of Cow Milk on Ethanol Stability. International Journal of Livestock Research, v.6, n.4, p.83-89, 2016. doi:10.5455/ijlr.20160325105132

ROMA JUNIOR, L.C.; SIMILI, F.F.; PAZ, C.C.P.; SALLES, M.S.V.; GONÇALVES, A.C.S.; MACHADO, P.F. Quality of milk protein in dairy farms from Southeastern region of Brazil. International Journal of Environmental & Agriculture Research, v.2, n.11, p.52-58, 2016.

ROSA, P.P.; ZANELA, M.B.; RIBEIRO, M.E.R.; FLUCK, A.C.; ANGELO, I.D.V.; FERREIRA, O.G.L.; COSTA, O.A.D. Fatores etiológicos que afetam a qualidade do leite e o Leite Instável Não Ácido (LINA). Revista Electrónica de Veterinaria, v.18, n.12, pp.1-17, 2017.

RUAS, J.R.M.; TORRES, C.A.A.; VALADARES FILHO, S.C.; PEREIRA, J.C.; BORGES, L.B.; MARCATTI NETO, A. Effect of Protein Supplementation at pasture on forage intake, weight gain and body condition in Nellore Cows. Revista Brasileira de Zootecnia. Viçosa, v.29, n.3, p.930-934, 2000

SILVA, D.A.; ROCHA JÚNIOR, V.R.; RUAS, J.R.M.; SANTANA, P.F.; BORGES, L.A.; CALDEIRA, L.A.; REIS, S.T.; MENEZES, J.C.; LANNA, D.P.D. Chemical and fatty acid composition of milk from crossbred cows subjected to feed restriction. Pesquisa Agropecuária Brasileira, v.54, e000051, 2019. DOI: https://doi.org/10.1590/S1678-3921.pab2019.v54.00051.

SHOOK, G.E.; BAMBER-KIRK, R.L.; WELCOME, F.L.; SCHUKKEN, Y,H.; RUEGG, P.L. Relationship between intramammary infection prevalence and somatic cell score in commercial dairy herds. Journal of Dairy Science, v. 100, p. 9691-9701, 2017

SOUZA, H.P.M.; ROMERO, N.B.; ROSA, C.C.B. Ocorrência do leite instável não ácido (LINA) na região norte do Mato Grosso. Revista do Instituto de Laticínios Cândido Tostes, v.71, n.1, p.38-42, 2016. DOI: 10.14295/2238-6416.v70i1.513

TROCH, T.; LEFÉBURE, É.; BAETEN, V.; COLINET, F.; GENGLER, N.; SINDIC M. Cow milk coagulation: process description, variation factors and evaluation methodologies. A review. Biotechnology, Agronomy and Society and Environment, v.21, p.1-12, 2017.

VOGES, J.G.; FELIPUS, N.C.; CANABARRO, L.O.; KNOB, D.A.; KAZAMA, D.C.S.; THALER NETO, A. Relação da infraestrutura da propriedade e alimentação dos animais na ocorrência de leite instável não ácido no planalto norte de Santa Catarina. Ciência Animal Brasileira, v.19, p.1-13, e-50399, 2018. DOI: 10.1590/1809-6891v19e-50399