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INTERRELATIONSHIPS BETWEEN MILK CONSTITUENTS, PROTEIN PROFILE, GENOTYPES OF KAPPA-CASEIN GENE AND ETHANOL STABILITY OF COW'S MILK

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ABSTRACT

This study aimed to withstand some factors that may be associated with and affected the alcohol instability of milk. Both individual milk and EDTA whole blood were collected from 162 cows among 4 Holstein dairy farms that suffered from their milk rejection because of milk ethanol instability. Milk samples were subjected to the measurement of milk components, pH, somatic cell count, and total bacterial count. Thirty milk samples were subjected to the detection of AFM1. Three mixed rations from each farm were subjected to the detection of AFB1. Blood samples were subjected to genotyping of the kappa-casein gene. About 72.2% and 27.8% of samples were recorded as ethanol-stable and unstable milk. A significant reduction was observed in the protein, fat, TS, and pH of ethanol unstable versus stable milk while a significant increase in salts, SCC, and TBC was observed. A significant difference was observed in AFM1 in stable versus unstable milk. The concentration of AFB1 ranged from 18.58 ppb up to 34.58 ppb. A significant difference in protein fractions was observed. Genotyping of the kappa-casein revealed that 51, 87, and 24 cows were AA, AB, and BB genotypes. Significantly higher fat and TS percentages and a significantly lower TBC in animals with BB genotypes ($p \leq 0.05$) versus those of both AA and AB genotypes were detected. In conclusion, continuous monitoring of different milk composition parameters, udder health represented by TBC and SCC and aflatoxin load in animal feed can be the first steps toward the production of ethanol-stable milk.

Keywords: Ethanol stability, cow milk, milk constituents, kappa-casein genotypes, aflatoxins

INTRODUCTION

The term "stability" refers to milk's capacity to withstand heat treatment with little to no industrial coagulation. Milk stability is a complex phenomenon regulated by several factors such as milk pH, acidity, casein micelle makeup, and calcium ions concentration (Lewis and Deeth, 2009; Fischer *et al*., 2012; Horne, 2015).

Numerous investigations have reported a significant frequency of unstable milk. It caused the dairy sector to suffer severe financial losses, especially in nations where

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the primary method of processing raw milk is the ultra-high temperature (UHT) (Fischer *et al*., 2012; Brasil *et al*., 2015).

It has been possible to assess milk stability using tests including ethanol, boiling, sedimentation, viscosity, and coagulation time. In many nations, the platforms at dairy farms and plants conduct ethanol tests to certify the stability of milk. Milk that fails the ethanol test is deemed unfit for use in industrial processes that require heating (Brasil, 2011).

Testing of milk stability to alcohol is mostly used to identify milk samples with high bacterial contamination, since poor sanitation practices and preservation during primary production led to decreased stability following heat treatment.

However, even if the acidity is less than 0.18 g lactic acid/100 ml milk, alcohol precipitati on of protein can still happen. Here, milk is not referred as unstable acid, which is an issue for dairy herds and/or the dairy industry. Since the same room for proc essing milk without high acidity is either ign ored or underappreciated by the industry, the loss of protein stability is a symptom of this issue, which impacts all chain links (Brasil *et al.*, 2015).

The reasons that predispose to the production of unstable milk are unknown. Several factors linked to metabolic and/or nutritional diseases, digestive difficulties, as well as genetic and environmental variables that can alter milk synthesis and milk components, have been identified in previous investigations (Martins *et al*., 2018).

Kappa-casein comprises up to 12% of the total bovine milk casein. An overall agreement about the relevance of casein gene variation to milk protein coagulation properties was recorded (Kyselová *et al*., 2019). However, studies concerning its relation to alcohol stability are limited and showed conflicting results (Botaro *et al*., 2009).

Aflatoxins are typically found in food when it is humid and warm, and are largely produced by the Aspergillus genus (Smith *et al.*, 2016). In the animal metabolic system, aflatoxin B1 (AFB1) was converted to aflatoxin M1 (AFM1). Then, it appears in milk and increases disease susceptibility (Giovati *et al*., 2015). Probably the most well-known mycotoxins are these two kinds of aflatoxin. Numerous studies have shown that dairy ruminants' immune systems, ability to acquire weight, and reproductive health are impacted by diets high in aflatoxins (Battacone *et al.*, 2005; Xiong *et al*., 2015). When determining the risk that mycotoxins are posed to dairy cows, it is crucial to study both macro-indicators (milk composition and production) and micro-indicators (bio-fluids biomarkers) of mycotoxicity. This is because AFB1 had a biochemical impact on animal health and may have metabolic markers in rumen fluid, milk, and blood (Wang *et al*., 2019).

Several complaints of milk ethanol instability have recently been reported in various Egyptian dairy farms, leading to its rejection or reevaluation by dairy factories. Therefore, the goal of this study was to withstand some selected elements from the multi-factorial causes of milk alcohol instability. These factors included milk parameters, milk protein fractionation profile, the genotype of the kappa casein gene, and the level of aflatoxin in both dairy milk (AFM1) and dairy diets (AFB1).

MATERIALS AND METHODS

Collection of samples:

Both composite individual milk and EDTA whole blood samples were collected simultaneously from 162 cows among four Holstein dairy cow farms that suffered from rejection or reevaluation of their bulk tank milk because of ethanol unstable milk (using alcohol precipitation test). After finishing

milking each animal, milk was homogenized and 50 ml was aseptically collected, maintaining the individuality of the samples, and transferred to the laboratory under refrigeration in ice-cooling bags. In the laboratory, each milk sample was divided into several parts for subsequent studies. Also, 3 randomly selected rations from each farm were collected to be later subjected to quantitative detection of aflatoxin B1 in the animal feed by ELISA technique.

Ethanol stability test

Each milk sample was subjected to an alcohol precipitation test by mixing equal volumes of milk and 70% ethanol, according to Rathnayake *et al*. (2016). The results were recorded according to the formation of clots on the wall of the test tubes. Milk was graded stable milk if no clots were formed, or unstable if minute clots were observed.

Measurement of milk components

Milk samples were analyzed to measure the percentages of protein, fat, lactose, solid not fat (SNF), total solids (TS), and salts using Lactoscan SLP (Bulgaria).

Measurement of somatic cell count and pH of milk

SCC of all milk samples was determined using the Nucleocounter SCC-100 (Chemometric Nucleocounter Family, Denmark). The pH was determined by a precision-calibrated pH meter PHS-3B (China).

Measurement of total bacterial count

An aliquot of 1 ml of each milk sample was serially diluted to enumerate the total bacterial count using the standard plate pouring method, according to National Standard Method (2005).

Determination of aflatoxin M1 in milk using ELISA kit

Only 30 milk samples; 15 from ethanol-unstable milk and 15 from ethanol-stable milk were randomly selected for this test. Aflatoxin M1 concentration in the selected milk samples was measured using a commercial ELISA kit (Helica Biosystems Inc., Santa Ana, CA, USA; catalog no. 961AFLM01M-96). The sample preparation was performed following the manufacturer's instructions.

Determination of aflatoxin B1 in ration using ELISA kit

Twelve mixed rations (3 from each farm) were subjected to quantitative detection of AFB1 in animal feed by ELISA technique. The sample preparation and measurement procedures were based on the manufacturer's instruction of ELISA kit recommendations (Helica Biosystems Inc., Santa Ana, CA, USA catalog no. 981AFL01M-96). Once the reaction was terminated, the absorbance was measured at the wavelength of 450 nm by using the ELISA reader (E_{max}, USA). The aflatoxin B1 content was calculated according to the reference curve.

Fractionation of milk proteins using SDS-PAGE

The skimmed milk was prepared from milk samples by centrifugation at 10000 rpm at -4°c for 15 minutes for removal of fat layer (Barbosa *et al*., 2012) and denatured in 15% sodium dodecyl sulphate-30% polyacrylamide separating gel electrophoresis according to the method of Laemmli, (1970) using mini-protein II electrophoresis cell (Bio-Rad). Electrophoresis was performed at 50 volts for 4 hrs. The electrophoretic patterns of structural proteins were matched with the full-range molecular weight protein marker ranging from 3.5 to 240 KDa (Gene Direx, Cat. No. PM008-0500). A sharp XJ-330 flat-bed scanner was used to take pictures of the gels, and Amersham Pharmacia Biotech Image Master 2-D Elite software was used to analyze the protein profiles in the images.

Genotyping of kappa casein gene using PCR-RFLP

Using the QIAamp Blood Mini Kit-Cat, No. 51104 (Qiagen, Germany), DNA was extracted from a 200 µl EDTA whole blood

sample in accordance with the manufacturer's instructions. Amplification of the kappa casein gene was performed according to Othman (2005) using K1 (5'-CACGTCACCCACACCCACATTTAT C-3') and K2 (5'-TAATTAGCCCATTTCGCCTTCTCTGT-3') primers. The reaction was conducted in SimpliAmp. thermal cycler, Thermo Fisher Scientific, Germany). PCR conditions begin with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. A final extension step at 72°C for 10 min was followed. After amplification confirmation using agarose gel electrophoresis, the digestion of PCR products using *Hind* III was performed according to manufacturing manuals (Thermo Scientific, Lithuania). Genotypes were identified according to Mitra *et al*. (1998), who revealed that the bovine AA genotype lacks the restriction site for Hind III in the 379 bp segment. As a result, it stays undigested and only generates one fragment of 379 bp. The BB genotype has a single restriction site, yielding two fragments of 225 and 154 bp. The presence of three fragments of 379, 225, and 154 bp was used to determine the AB genotype.

Statistical analysis

The Online Gene Calculator was used to determine the genotype and allele frequencies of SNP polymorphism, as well as their departure from Hardy-Weinberg equilibrium using the $\chi 2$ test (https://gene-calc.pl/hardy-weinberg-page). Analysis of the variance of the obtained results was performed according to Sendecor and Cochran (1989). Values were expressed as mean ± SE. Statistical comparisons between means of different groups were made with a completely randomized one-way ANOVA Student-Newman-Keuls test by COSTAT program version 1. A probability of a "*P*" value of < 0.05 was assumed for statistical significance.

RESULTS

Out of 162 individual cow's composite milk, 117 (72.2%) and 45 (27.8%) were recorded as ethanol stable and unstable milk, respectively. As declared in Table 1, highly significant differences ($p<0.05$) were observed in protein, fat, salt percentages, pH, SCC, and TBC of milk samples from stable and unstable milk samples. Also, a significant difference was noticed in TS%. There was a significant reduction in protein, fat, TS, and pH of unstable milk compared to stable milk (2.69±0.18, 1.89±0.24, 10.63±0.35 and 6.44±0.13 versus 3.36±0.12, 3.38±0.37, 11.87±0.24 and 6.69±0.07, respectively). On the contrary, a significant increase in salts, SCC, and TBC of unstable milk was also observed (0.72±0.029, 625±68 $x10^3$ and 339±26 $x10^3$ versus 0.65±0.017, 178±16 $x10^3$ and 64±5 $x10^3$, respectively). On the other hand, no significant difference was detected in lactose and SNF percentages.

Table 1: Difference between ethanol stable and unstable milk on level of milk analysis parameters

Ethanol stability of milk	Protein %	Fat%	Lactose %	SNF%	T.S.%	Salt%	pH	SCC×10^3 cells/ml	TBC×10^3 CFU/ml	AFM1 ppt
Stable milk	3.36 ±0.12	3.38 ±0.37	4.66 ±0.31	8.49 ±0.62	11.87 ±0.24	0.65 ±0.017	6.69 ±0.07	178 ±16	64 ±5	0.126± 0.25
Unstable milk	2.69 ±0.18***	1.89 ±0.24***	4.7 ±0.23n.s	8.74 ±0.71n.s	10.63 ±0.35*	0.72 ±0.029***	6.44 ±0.13***	625 ±68***	339 ±26 ***	4.006± 0.264***

Values: Means ±SE
n.s: non-significant, ***highly significant, **moderately significant, * low significant

Considering the aflatoxin AFM1 and AFB1 levels in the cow's milk and feed, random samples were collected from the milk and ration of the four farms (30 milk samples and 12 feed samples). A highly significant lower concentration of AFM1 was observed in the alcohol stable milk when compared with the unstable milk samples (0.126±0.025 versus 4.006±0.264*** ppt). The concentration of AFB1 in ration samples ranged from 18.58 ppb up to 34.58 ppb with an overall mean of 26.14 ± 1.28 ppb.

As presented in Table 2, there was a significant difference in the level of milk protein fractions between ethanol stable and unstable milk samples. Both albumin and globulin were increased significantly (p<0.05) in ethanol unstable milk compared with stable milk (7.08±0.05 and 7.28±0.43 versus 6.8±0.03 and 5.13±0.23, respectively), while casein content decreased significantly in ethanol unstable milk versus stable milk (9.96±0.44 versus 17.84±0.52, p≤0.005).

Table 2: Milk protein profile fractionation of both ethanol-stable and unstable milk samples.

Skimmed milk protein fractions (%)	Ethanol stable milk (No.=15)	Ethanol unstable milk (No.=15)
Albumin	6.8±0.03	7.08±0.05*
Globulin	5.13±0.23	7.28±0.43**
Casein	17.84±0.52	9.96±0.44***

Figure (1): Showed the fractionation of skimmed milk protein.

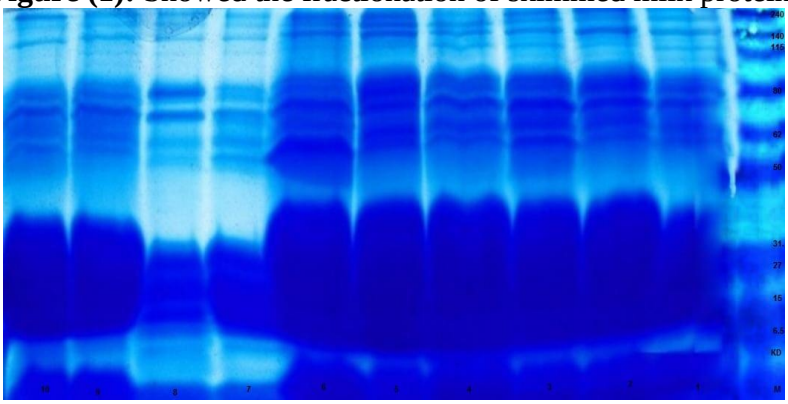

Figure 1: Electrophoretic protein profiles of different skimmed milk samples separated on 15% sodium dodecyl sulphate-30% polyacrylamide separating gel.
M: Full-range molecular weight protein marker 6.5 -420 KD.
Lanes (1-6): representative ethanol stable milk samples.
Lanes (7-10): representative ethanol unstable milk samples.

The genotype of the *k*-casein gene of the 162 cows was identified using RFLP-PCR. As shown in table 3, among the screened 162 cows, 51, 87, and 24 cows were of the AA, AB, and BB genotypes, respectively. The number of cows of each genotype among cows with stable and unstable milk was also depicted in the same table.

Table 3: The frequencies and percentages of different *k*-casein genotypes in the screened cows with ethanol stable and unstable milk

***K*-casein Genotypes (No.)**	**Cows with stable milk samples (No.=117)**		**Cows with unstable milk samples (No.=45)**	
	No.	**%**	**No.**	**%**
AA (51)	33	64.71	18	35.29
AB (87)	60	68.97	27	31.03
BB (24)	24	100	0	0

The number of observed and expected genotypes of kappa casein SNP and Chi-square (χ^2) value are shown in table 4. Chi-square (χ^2) value indicated that the numbers of the expected and observed genotypes were very close, and the population was considered in Hardy-Weinberg equilibrium at the level of significance 0·05.

Table 4: Allele frequency, observed and expected genotype frequencies for kappa-casein gene, and chi-square ($\chi 2$) values for Hardy-Weinberg equilibrium test at $p<0.05$.

Total No.		**Genotype Frequency**			**Allele frequency**		**χ^2**
		AA	AB	BB	A	B	
162	Observed	51	87	24	0.5833	0.4167	1.778
	Expected	55.13	78.75	28.13			

As shown in Table 5, data from milk analysis suggested significantly higher fat and TS percentages (3.24±0.2 and 11.84±0.37, respectively, versus 2.67±0.1 and 10.97±0.33, respectively for AA and 1.89±0.3 and 10.69±0.29, respectively, for AB) and a significantly lower TBC (110±12 x10^3 versus 330±18 x10^3 and 288±23 x10^3) in animals with BB genotypes ($p\leq0.05$) versus those of both AA and AB genotypes, respectively. Other milk parameters showed no significant difference between animals with different genotypes.

Table 5: Correlation between *k*-casein genotypes and milk parameters

***k*-casein Genotypes**	**Protein%**	**Fat%**	**Lactose %**	**SNF%**	**T.S%**	**Salts%**	**pH**	**SCC×10^3 cells/ml**	**TBC×10^3 CFU/ ml**
AA	3.05± 0.4	2.67± 0.1**	4.4± 0.24	8.3± 0.52	10.97± 0.33*	0.68± 0.03	6.5± 0.21	397± 24	330± 18**
AB	2.94± 0.3	1.89± 0.3**	4.6± 0.31	8.8± 0.44	10.69± 0.29*	0.65± 0.02	6.6± 0.42	388± 18	288± 23**
BB	3.31± 0.5	3.24± 0.2	4.6± 0.32	8.6± 0.32	11.84± 0.37	0.67± 0.04	6.3± 0.33	354± 31	110± 12

DISCUSSION

Technological properties and the value of cow milk considerably influence the dairy industry. Many factors can affect these technological properties, like; environmental, genetic, seasonal, different feed rations, the health aspects of milk production performance, and

milk quality (Migliorati *et al*., 2017; Beux *et al*., 2018; Ghelichkhan *et al*., 2019; Čítek *et al*., 2020). This study aimed to spotlight some of these technological properties that may have negative effects on the dairy cows' milk industry with the first quality testing at the platform using alcohol or ethanol stability testing of raw milk. To do this, 162 raw individual 'cows milk samples were collected from 4 dairy farms in response to complaints of alcohol instability of their milk and rejection or revaluation of their raw milk. All samples were subjected to an ethanol stability test. Among the examined samples, 45 were ethanol unstable milk with a percentage of 27.8%, while the rest were ethanol stable milk (72.2%). In this aspect of work, nearly similar percentages of unstable milk, including 30.49, 30.2, and 32% were previously recorded by Botaro *et al*. (2009), Fagnani *et al*. (2014), and Fagnani *et al*. (2018), respectively. A slightly lower percentage (23%) was reported by Oliveira *et al*. (2013). The highest reported percentage of ethanol unstable milk was higher than 50% (Zanela *et al*., 2009).

Considering the comparison between ethanol stable and unstable milk on the level of different milk parameters, it was clear that protein, fat, TS, and pH were significantly decreased in unstable milk versus stable one. On the contrary, salts, SCC, and TBC were significantly increased in unstable milk. However, there was no discernible variation in the percentages of lactose or SNF levels. Machado *et al*. (2017) reported better chemical composition of high milk ethanol stability in comparison to ethanol unstable milk. Also, Chavez *et al*. (2004) declared that much attention must be paid to other important variables influencing milk alcohol stability, including its composition. On the contrary, Citek *et al*. (2020) reported that the significant difference between stable and unstable milk on the level of milk components is scarce. Therefore, we think that any alteration in milk composition can act as a predisposing factor to ethanol instability. Moreover, the increase in TBC and SCC may lead to release of high levels of proteolytic and lipolytic enzymes, which in turn can degrade protein and fat in milk, and therefore both protein and fat content in milk decreased. Also, as a result of the increased TBC, a change in the pH occurs due to excessive bacterial metabolism excretions. Additionally, the increased level of both may also change the permeability of the mammary gland epithelial lining and their tied junction, which led to the salt imbalance, especially due to bacterial toxins.

Rathnayake *et al*. (2016) also reported a significant decrease in the pH of unstable milk versus stable milk. Tsioulpas *et al*. (2007) concluded that when milk pH was below 6.55, milk ethanol stability decreased significantly, whereas samples that had a pH above 6.85 showed a very high alcohol stability.

Contrary to the obtained results, Rathnayake *et al*. (2016) reported no significant difference considering protein, SNF, TS, and TBC between stable and unstable milk samples. Also, Fagnani *et al*. (2018) found no difference in the percentages of fat, protein, TS, SCC, and TBC between stable and unstable milk. On the same line, Hanuš *et al*. (2019) found a significant relationship between the stability of raw cow milk and several milk indicators, such as fat, crude protein content, TS, and fat/lactose ratio. This might enhance the accuracy of raw material selected estimation and thermo-stability prediction.

Milk salt concentration is essential for the milk's technological properties. In milk, cations make up most of the salts, followed by anions. The anions are phosphate, citrate, and chloride, whereas the cations are calcium, magnesium, potassium, and sodium. They can either be disseminated in milk serum as free ions or ion pairs, or in colloidal calcium phosphate nanoclusters attached to caseins. The distribution of salts, particularly the amount of calcium phosphate distributed between the micelle of casein and the serum phase, significantly affects the casein micelles stability and structure (Lucey and Horne, 2009; Holt, 2011; Dalgleish and Corredig, 2012; de Kruif *et al*., 2012). In the current study, milk salts were significantly lower in ethanol-stable milk, when compared to unstable milk. This may assure the role played by salts in the process of milk stability. In this concern, very little literature studied the relationship between total milk salts and ethanol stability, as most researchers concentrated on the cationic portion of these salts. However, Bijl *et al*. (2013) discovered that the first family of correlations in their analysis comprised multivalent micellar salts, which were closely associated with the protein content of the milk recognized to be correlated to the process of

milk ethanol stability in the current literature. In the far past, Donnelly and Horne (1986) suggested that a high salt balance ratio during late and early lactation was an important contribution to this milk ethanol stability. Additionally, Kailasapathy *et al*. (2008) mentioned a few elements crucial for milk proteins' ability to withstand heat, including milk pH, salt concentration, lactose, protein, the season, lactation, and cow health.

Somatic cell count and TBC were reported to be both health and hygiene indicators for dairy cows (Hanuš *et al*., 2021). In discussing the difference between ethanol stable and unstable milk with SCC and TBC in this study, a significant increase in both SCC and TBC in the unstable milk was observed, as shown in table (1). These findings agreed with those of Machado *et al*. (2017), who noted that ethanol milk stability decreased significantly when SCC of milk increased to 790000:10^6 cells/ml, and when TBC exceeds 250,000 CFU/ml. Milk with high stability is defined by lower values of SCC and TBC but higher values for pH, lactose, and protein in comparison to milk with low stability. Also, the results of Oliveira *et al*. (2013) suggested that the high SCC may be involved as a causal factor for the high incidence of ethanol unstable milk as the last showed higher SCC as well as lower casein contents which approved our results. Lately, Hanuš *et al*. (2021) cleared that, milk stability values suggested similar seasonal dynamics with the SCC and TBC and opposed composition patterns to crude protein, fat, SNF, and TS content.

Several researchers explained the negative correlations between milk ethanol stability and both SCC and TBC as Chavez *et al*. (2004); Bueno *et al*. (2008); Lewis and Deeth (2009) and Horne (2015); they illustrated that casein micelle instability or its low content, high salt and chlorine levels as well as high SCC values were related to low milk stability brought on by the proteolysis process. Furthermore, elevated SCC in milk is associated with the lysis of casein fractions, particularly αS- and β-casein, because of the released proteolytic enzymes from the somatic cells. Additionally, the potential of milk as a raw material for product manufacturers as well as the industrial output of milk were both strongly impacted by the changes in protein fractions of milk brought on by high SCC. The casein fraction was primarily responsible for these changes (Fernandes *et al*., 2008 and Ramos *et al*., 2015). Similarly, high TBC values were associated with low milk stability, which was likely caused by both proteolysis and lactose degradation which consequently increased ionic calcium concentrations, decreased pH and calcium phosphate values, and destabilized casein micelles (Bueno *et al*., 2008; Lewis and Deeth, 2009 and Machado *et al*., 2017). However, Kolling (2012) did not find any relationship between both SCC and TBC and milk ethanol stability.

Numerous studies noted additional variables that can influence milk composition and milk ethanol stability, such as the nutritional deficit, which is known to be a major contributor to milk proteins instability as measured by an alcohol test (Zanela *et al*., 2006; Barbosa *et al*., 2012; Stumpf *et al*., 2013). According to Stumpf *et al*. (2013), feed restriction may cause mineral inbalance that reduces milk ethanol stability and increases the permeability of mammary gland cell tight junctions. Heat stress, reduction in dietary cation-anion differences, and ruminal and metabolic acidosis have all been linked to decreased milk ethanol stability, according to Marques *et al*. (2011) and Martins *et al*. (2015). In Brazil and other nations that regularly utilize ethanol tests for milk evaluation, the incidence of unstable non-acid milk can therefore be strongly linked to dietary imbalance, digestive issues, as well as hereditary and environmental circumstances, but not to subclinical intra-mammary infections (Martins *et al*., 2018).

Aflatoxin B1 is one of the quality indicators for animal diet. It is also excreted in the milk as aflatoxin M1 (Giovati *et al*., 2015). Considering the relation between milk ethanol stability and the presence of aflatoxin, our results cleared that the level of AFM1 in unstable milk was significantly higher than that in ethanol stable milk (4.006±0.264 versus 0.126±0.025). Also, the level of aflatoxin B1 in the examined rations that were collected from farms suffering from unstable milk ranged from 18.58 up to 34.58 ppb with an overall mean of 26.14 ± 1.28 which is considered a high level. Few authors recorded AFB1or M1 to have either direct or indirect effects on milk ethanol stability. They may influence milk composition of stable and unstable milk which agree with our results as cleared in table 1. Likewise, the obtained results, Křížová *et al*. (2016) illustrated that ethanol

stability, as well as milk indicators like fat, pH, and fat/crude protein ratio, were influenced by the mycotoxin load of herd's feed including aflatoxins. Moreover, when the overall level of mycotoxin load was relatively low; there was no clear effect on milk characteristics which supported our results.

However, Queiroz *et al*. (2012) and Intanoo *et al*. (2020) reported that when cows exposed to naturally contaminated diets contained 22.28 µg/kg of AFB1 resulted in a significant drop in both protein concentration and fat yield of milk. Furthermore, Cha *et al*. (2021) illustrated that there were no significant differences in milk components (milk fat, protein, lactose, and SCC) of the dairy cows fed with the moderate risk level of (8 g/kg of diet dry matter) AFB1with or without adsorbents. Moreover, Xiong *et al*. (2015) and Gonçalves *et al*. (2017) proclaimed that multiple factors worked behind the aflatoxin M1 excretion amount into raw milk like; AFB1 cow's dietary levels, stage of lactation, milk yield, mammary gland condition, and individual receptivity. Previously, Kelly *et al*. (1982) attributed the instability of milk to seasonal variability in feed ration composition that was seen in milk produced between November and March. Dairy cows spend this time indoors, thus they found that the milk had less thermal stability than milk from other times of the year (April to October).

In the current study, significant differences (p <0.05) were recorded between all the skimmed milk major protein fractions including total lactalbumin, lactoglobulin, and casein between ethanol stable and unstable milk as cleared in table 4. Both albumin and globulin percentages were increased significantly (p<0.05) in ethanol unstable milk versus stable milk. On the contrary, casein content decreased significantly in alcohol unstable in comparison to stable milk (9.96±0.44 versus 17.84±0.52, p≤0.005). Similarly, Fagnani *et al*. (2018) reported a relationship between α-lactalbumin and ethanol stability, leading them to conclude that α-lactalbumin was a key component for the osmotic balance of milk, and thus its ethanol stability. By contrast, Botaro *et al*. (2009) and Fagnani *et al*. (2018) recorded that their investigations showed no differences between the stable and unstable groups for other protein fractions, including casein and β-lactoglobulin. Meanwhile, Barbosa *et al*. (2012) indicated that the correlation between milk ethanol stability and milk protein fractions could be mainly influenced by κ-casein and β-lactoglobulin, which are related to micellar stability, as well the obtained results agreed with this statement. Caseins are milk proteins released by mammary gland cells. They make up roughly 78–82 percent of the proteins in cow's milk (Dalgleish and Morris (1988). The observed decrease in casein content of the investigated unstable milk can be explained differently. Sinagaa *et al*. (2017) related this observation to the adjustment of milk pH to 6.0, which led to a decrease in casein micelles size, accompanied by an increase in soluble Ca, consequently a decrease in milk ethanol stability. Also, Oliveira *et al*. (2013) added that alcohol-unstable milk samples showed non-significant lower percentages of α and β-casein than stable milk, and it is required to understand if the changes in casein ratio of elevated SCC milk could be correlated with the existence of unstable milk. However, these samples revealed lower amounts of casein and higher SCC. Moreover, calcium phosphate distribution between the casein micelle and serum phase has a significant impact on the stability and shape of casein micelles (Dalgleish and Corredig, 2012). Overall, 70%, 50%, 30%, 10% of calcium, inorganic phosphate, magnesium, and citrate respectively in milk are in the casein micelle and are essential for its stability (Holt, 2004; Walstra *et al*., 2005; Farrell *et al*., 2006). Finally, when correlating TBC and casein, high TBC levels caused proteolytic bacterial enzymes to act primarily on casein, destabilizing the casein micelles and causing milk to coagulate (Recio *et al*., 1996).

To explore the effect of different genotypes of the κ-casein gene on milk stability and milk composition, all animals were genotyped for their κ-casein using the PCR-RFLP method. As shown in Table 3, out of the 162 samples, 51, 87, and 24 were of the AA, AB, and BB genotypes, respectively. The number of observed and expected genotypes and their Chi-square value were depicted in table 4. At the significance threshold of 0.05, the Chi-square value showed that the numbers of observed and expected genotypes were nearly similar, and the population was regarded as in the Hardy-Weinberg equilibrium. This might be explained by the large proportion of heterozygous individuals of genotype AB, which could keep the population's allele frequencies evenly

distributed. No significant association was obtained between kappa casein genotypes and milk alcohol stability. However, it was interesting to notice that none of the animals suffered from unstable milk were of BB genotype. Similarly, Botaro *et al*. (2009) showed no correlation between the genotypes of *κ*-casein and the prevalence of milk ethanol stability. Thus, the noted variations in the ethanol stability test and the milk stability test were merely the result of random variation and were unrelated to the genotypes AA, AB, and BB under study. In the present study, there was no significant association between *k*-casein genotypes and most milk parameters except fat, TS, and TBC, which showed a significant association with *k*-casein genotypes ($P<0.05$) whereas animals of the BB genotype showed a higher level of these parameters than the animals of either AA or AB genotypes. Like the present findings, Botaro *et al*. (2009) reported that *k*-casein polymorphism had no effect on the milk's physicochemical properties. However, cows with the BB genotype had higher milk fat content than those with the AA genotype. While studies on the relationship between genetic variations in *κ*-casein and milk composition by Molina *et al*. (2006) and Hallen *et al*. (2008) revealed somewhat divergent findings. While studies on the relationship between genetic variations in k-casein and milk composition by Molina *et al*. (2006) and Hallen *et al*. (2008) revealed somewhat divergent findings. Additionally, alterations in milk protein contents brought by the *k*-casein genotypes A and B were observed by Ng-Kwai-Hang *et al*. (1990). While previous researchers have observed greater casein and protein concentrations in cow's milk with the allele B of the *k*-casein gene, Haenlein *et al*. (1987) noticed no influence of *k*-casein variations on milk crude protein. These conflicting results can be attributed to the difference in the breeds and numbers of studied animals. Imafidon *et al*. (1991) and Robitaille (1995) showed that milk from cows of *κ*-casein AA genotype was more stable in the ethanol test, in contrast to our findings, whereas Paterson *et al*. (1999) discovered increased stability in milk with the *k*-casein BB genotype, which was consistent with the obtained findings.

CONCLUSION

In this study, the variability of ethanol-stable milk was significantly influenced by several parameters. Partial quantification and explanation of these factors were the aims of this paper. This wide range of variations aided in explaining the discrepancies in findings between studies that examined milk stability to ethanol in relation to milk composition, health, and hygiene indicators (expressed in both SCC and TBC), one of the feed defects (AFB1), and various milk protein fractions.

Generally, better milk chemical composition, accurate health, hygiene indicators, and good animal feed resulted in milk with high ethanol stability and vice versa. Therefore, continuous monitoring and following up of different milk composition parameters, udder health represented by TBC and SCC, and aflatoxin load in animal feed can be the first step toward production of ethanol stable milk.

REFERENCES

Barbosa, R.S.; Fischer, V.; Ribeiro, M.E.; Zanela, M.B.; Stumpf, M.T. and Kolling, G.J. (2012): Electrophoretic characterization of proteins and milk stability of cows submitted to feeding restriction. Pesq.Agropec. Bras., 47:621-628.

Battacone, G.; Nudda, A.; Palomba, M.; Pascale, M.; Nicolussi, P. and Pulina, G. (2005): Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. J. Dairy. Sci., 88, 3063–3069.

Beux, S.; Cassandro, M.; de Lima, V.A.; Zielinski, A.A.F.; Nogueira, A. and Waszczynskyj, N. (2018): Seasonal effect on milk composition, somatic cell content and milk coagulation properties of Italian Holstein-Friesian cows. Emir. J. Food Agr., 30:998-1005.

Bijl, E.; Van Valenberg, H.J.F.; Huppertz, T. and Van Hooijdonk ACM (2013). Protein, casein, and micellar salts in milk: Current content and historical perspectives. J. Dairy Sci., 96: 5455–5464.

Botaro, B.G.; de Lima, Y.V.R.; Cortinhas, C.S.; Silva, L.F.P.; Rennó, F.P. and dos Santos, M.V. (2009): Effect of the kappa-casein gene polymorphism, breed and seasonality on physicochemical characteristics, composition and stability of bovine milk. R. Bras. Zootec., 38 (12): 2447-2454.

Brasil, R.B.; Nicolau, E.S. and Silva, M.A.P. (2015): Not acid unstable milk and factors affecting the stability of milk.Ciência Animal, 25 (4): 15-26.

Brasil, (2011): Instrução Normativa n. 62, de 29 de dezembro de 2011. Alteração do caput da Instrução Normativa MAPA n. 51, de 18 de setembro de 2002. Diário Oficial da União, Brasília, 29 dez 2011.

Bueno, V.F.F.; Mesquita, A.J.; Oliveira, A.N.; Nicolau, E.S. and Neves, R.B.S. (2008): Total bacterial count: relationship to milk composition and period of the year in Goiás State, Brazil. Revista Brasileira de Ciências Veterinárias, 15 (1): 40-44.

Cha, M.; Wang, E.; Hao, Y.; Ji, S.; Huang, S.; Zhao, L.; Wang, W.; Shao, W.; Wang, Y. and Li, S. (2021): Adsorbents reduce aflatoxin M1 residue in milk of healthy dairy cow exposed to moderate level aflatoxin B1 in diet and its exposure risk for humans. Toxins, 13 (9): 665 (14p).

Chavez, M.S.; Negri, L.M.; Taverna, M.A. and Cuatrín, A. (2004): Bovine milk composition parameters affecting the ethanol stability. J Dairy Res., 71(2): 201-206.

Čítek, J.; Brzáková, M.; Hanusová, L.; Hanuš, O.; Večerek, L.; Samková, E.; Křížová, Z.; Hoštičková, I.; Kávová, T.; Straková, K. and Hasoňová, L. (2020): Technological properties of cow's milk: correlations with milk composition, effect of interactions of genes and other factors. Czech J. Anim. Sci., 65 (1): 13-22.

Dalgleish, D.G. and Corredig, M. (2012): The structure of the casein micelle of milk and its changes during processing. Annu. Rev. Food Sci. Technol., 3:449-467.

Dalgleish, D.G. and Morris ER (1988): Interactions between carrageenans and casein micelles: electrophoretic and hydrodynamic properties of the particles. Food Hydrocoll, 2: 311-320.

de Kruif, C.G.; Huppertz, T.; Urban, V.S. and Petukhov, A.V. (2012): Casein micelles and their internal structure. Adv. Colloid Interface Sci., 171-172: 36‑52.

Donnelly, W.J. and Horne, D.S. (1986): Relationship between ethanol stability of bovine milk and natural variations in milk composition. J. Dairy Res., 53: 23-33.

Fagnani, R.; Beloti, V. and Battaglini, A.P.P. (2014): Acid-base balance of dairy cows and its relationship with alcoholic stability and mineral composition of milk. Pesquisa Veterinária Brasileira, 34(5):398-402.

Fagnani, R.; de Araὺjo, J.P.A. and Botaro, B.G. (2018): Field findings about milk ethanol stability: a first report of interrelationship between α-lactalbumin and lactose. J. Sci. Food Agric., 98: 2787-2792.

Farrell, H.M.; Malin, E.L.; Brown, E.M. and Qi, P.X. (2006): Casein micelle structure: What can be learned from milk synthesis and structural biology? Curr. Opin. Colloid Interface Sci., 11(2): 135-147.

Fernandes, A.M.; Bovo, F.; Moretti, T.S.; Rosim, R.E.; Lima, C.G. and Oliveira, C.A.F. (2008): Casein fractions of ultra high temperature milk with different somatic cell counts. Pesquisa Agropecuária Brasileira, 43(1): 149-152.

Fischer, V.; Ribeiro, M.E.R.; Zanela, M.B.; Marques, L.T.; Abreu, A.S.; Machado, S.C.; Fruscalso, V.; Barbosa, R.S. and Stumpf, M.T. (2012): Unstable non acid milk: a solvable problem? Rev. Bras. Saúde Prod. Anim., 13(3): 838-849.

Ghelichkhan, M.; Amanlou, H.; Mahjoubi, E. and Patton, R.A. (2019): Solvent extracted soybean meal top-dressed on a fresh cow diet increased milk production, but not milk components, and decreased plasma non-esterified fatty acids. Czech J. Anim. Sci., 64(1): 26-40.

Giovati, L.; Magliani, W.; Ciociola, T.; Santinoli, C.; Conti, S. and Polonelli, L. (2015): AFM1 in milk: Physical, biological, and prophylactic methods to mitigate contamination. Toxins, 7(10): 4330-4349.

Gonçalves, B.L.; Gonçalves, J.L.; Rosim, R.E.; Cappato, L.P.; Cruz, A.G.D.; Oliveira, C.A.F.D.; Corassin, C.H. (2017): Effects of different sources of Saccharomyces cerevisiae biomass on milk production, composition, and aflatoxin M1 excretion in milk from dairy cows fed aflatoxin B1. J. Dairy Sci., 100 (7): 5701-5708.

Haenlein, GF.W.; Gonyon, D.S.; Mather, R.E. and Hines, H.C. (1987): Associations of bovine blood and milk polymorphisms with lactation traits - Guernseys. J Dairy Sci., 70(12): 2599-2609.

Hallen, E.; Wedholm, A.; Andren, A. and Lundén, A. (2008): Effect of betacasein, kappa-casein and beta-lactoglobulin genotypes on concentration of milk

protein variants. J. Anim. Breed Genet., 125(2): 119-129.

Hanuš, O.; Čítek, J.; Říha, J.; Samková, E.; Kučera, J.; Chládek, G.; Němečková, I.; Hasoňová, L.; Klimešová, M,; Roubal, P. and Jedelská, R. (2019): Seasonal correlations between heat stability and other raw bulk cow milk quality indicators. Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis, 35 (2):395-406.

Hanuš, O.; Kučera, J.; Samková, E.; Němečková, I.; Čítek, J.; Kopec, T.; Falta, D.; Nejeschlebová, H.; Rysová, L.; Klimešová, M. and Elich, O. (2021): Raw cow milk protein stability under natural and technological conditions of environment by analysis of Variance. Foods, 10(9): 2017; 15p.

Holt, C. (2004): An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. Eur. Biophys. J., 33(5): 421‑434.

Holt, C. (2011): Milk salts: their interaction with caseins. Encyclopedia of Dairy Sciences, Volume 3, 2nd ed. Pages 917-924. Academic Press, San Diego, CA.

Horne, D.S. (2015): Ethanol stability and milk composition. In: Advanced dairy chemistry. Volume 1B: Proteins: Applied aspects. 4th ed. McSweeney, P.L.H. and O'Mahony, J.A., eds. Springer, Cork.

Imafidon, G.I.; Ng-Kwai-Hang, K.F. and Harwalkar, V.R. (1991): Effect of genetic-polymorphism on the thermal-stability of beta-lactoglobulin and kappa-casein mixture. J Dairy Sci., 74(6):1791-1802.

Intanoo, M.; Kongkeitkajorn, M.B.; Suriyasathaporn, W.; Phasuk, Y.; Bernard, J.K. and Pattarajinda, V. (2020): Effect of supplemental *Kluyveromyces marxianus* and *Pichia kudriavzevii* on aflatoxin M1 excretion in milk of lactating dairy cows. Animals, 10 (4):709-11p.

Kailasapathy, K.; Chandan, R.C.; Kilara, A. and Shah, N.P. (2008): Chemical composition, physical and functional properties of milk and milk ingredients. In Dairy Processing and Quality Assurance, Eds.; John Wiley & Sons: Hoboken, NJ, USA; Chapter 4, pp. 75-103.

Kelly, P.; O'Keeffe, A.; Keogh, M. and Phelan, J. (1982): Studies of milk composition and its relationship to some processing criteria: III: Seasonal variation in heat stability of milk. Ir. J. Food Sci. Technol., 6(1): 29-38.

Kolling, G.K. (2012): Influência da mastiten aqualidade do leite e leitein stávelnão ácido emdiferentes quartos mamários. Dissertação (M.Sc.). Universidade Federal do Rio Grande do Sul. Porto Alegre, RS, Brasil.

Křížová, L; Hanuš, O.; Klimešová, M.; Nedělník, J.; Kučera, J.; Roubal, P.; Kopecký, J. and Jedelská, R. (2016): Chemical, physical and technological properties of milk as affected by the mycotoxin load of dairy herds. Arch. Anim. Breed., 59(2): 293-300.

Kyselová, J.; Ječmínková, K.; Matějíčková, J.; Hanuš, O.; Kott, T.; Štípková, M. and Krejčová, M. (2019): Physiochemical characteristics and fermentation ability of milk from Czech Fleckvieh cows are related to genetic polymorphisms of β-casein, κ-casein, and β-lactoglobulin. Asian-Australas J. Anim. Sci., 32(1):14-22.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature, 227: 680- 68.

Lewis, M.J. and Deeth, H.C. (2009): Heat treatment of milk. p. 168-204. In: Milk processing and quality management. Tamine, A. Y., ed. Wiley-Blackwell, Chichester, UK.

Lucey, J.A. and Horne, D.S. (2009): Milk salts: Technological significance. p. 351-390 in Advanced Dairy Chemistry: Volume 3: Lactose, Water, Salts and Minor Constituents. Springer-Verlag, New York, NY.

Machado, S.C.; Fischer, V.; Stumpf, M. and Stivanin, S.C.B. (2017): Seasonal variation, method of determination of bovine milk stability, and its relation with physical, chemical, and sanitary characteristics of raw milk. R. Bras. Zootec., 46(4):340-347.

Marques, L.T.; Fischer, V.; Zanela, M.B.; Ribeiro, M.E.R.; Stumpf, J.W. and Rodrigues, C.M. (2011): Milk yield, milk composition and biochemical blood profile of lactating cows supplemented

with anionic salt. R. Bras. Zootec., 40 (5): 1088-1094.

Martins, C.M.R.; Arcari, M.A.; Welter, K.C.; Netto, A.S.; Oliveira, C.A.F. and Santos, M.V. (2015): Effect of dietary cation-anion difference on performance of lactating dairy cows and stability of milk proteins. J. Dairy Sci., 98(4): 2650-2661.

Martins, C.M.R.; Gonçalves, J.L.; Alves, B.G.; Arcari, M.A. and Santos, M.V. (2018): Subclinical intramammary infection does not affect bovine milk ethanol stability. Braz. J. Vet. Res. Anim. Sci., São Paulo, 55 (2): 1-9.

Migliorati, L.; Boselli, L.; Pirlo, G.; Moschini, M. and Masoero, F. (2017): Corn silage replacement with barley silage in dairy cows' diet does not change milk quality, cheese quality and yield. J. Sci. Food Agric., 97(10): 3396-3401.

Mitra, A.; Schlee, P.; Krause, I.; Blusch, J.; Werner, T.; Balakrishnan, C.R. and Pirchner, F. (1998): Kappa-casein polymorphisms in Indian dairy cattle and buffalo: a new genetic variant in buffalo. Anim. Biotech., 9(2): 81-87.

Molina, L.H.; Kramm, J.; Brito, C.; Carrillo, B.; Pinto, M. and Ferrando, A. (2006): Protein composition of milk from Holstein–Friesian dairy cows and its relationship with the genetic variants A and B of kappa-casein and beta-lactoglobulin. Int. J. Dairy Tech., 59(3): 183-187.

National Standard Method (2005): Plate count test at 30 ºC. National Public Health Service for Wales, Health Protection, Agency, pp.1-11.

Ng-Kwai-Hang, K.F.; Monardes, H.G. and Hayes, J.F. (1990): Association between genetic polymorphism of milk proteins and production traits during three lactations. J. Dairy Sci., 73(12): 3414-3420.

Oliveira, C.A.F.; Lopes, L.C.; Rosim, R.E.; Fernandes, A.M. and Corassin, C.H. (2013): Composition, somatic cell count and casein fractions of ethanol unstable milks. Acta Scientiarum. 35(1): 153-156.

Othman, O.E. (2005): The identification of kappa-casein genotyping in Egyptian river buffalo using PCR-RFLP. Arab J. Biotech., 8 (2): 265-274.

Paterson, G.R.; Macgibbon, A.K.H. and Hill, J.P. (1999): Influence of kappa-casein and beta-lactoglobulin phenotype on the heat stability of milk. Int. Dairy J., 9(3-6):375-376.

Queiroz, O.C.M.; Han, J.H.; Staples, C.R. and Adesogan, A.T. (2012): Effect of adding a mycotoxin-sequestering agent on milk aflatoxin M1 concentration and the performance and immune response of dairy cattle fed an aflatoxin B1 contaminated diet. J. Dairy Sci., 95(10): 5901-5908.

Ramos, T.M.; Costa, F.F.; Pinto, I.S.B.; Pinto, S.M. and Abreu, L.R. (2015): Effect of Somatic Cell Count on Bovine Milk Protein Fractions. J Anal Bioanal Tech., 6(5): 269- 7p.

Rathnayake, R.M.C.S.; Mangalika, U.L.P.; Adikari, A.M.J.B. and Nayananjalie, W.A.D. (2016): Changes in compositional and keepingquality parameters ofcowmilk on ethanol stability. Int. J. Livest. Res., 6(4): 83-89.

Recio, I.; Frutos, M.; Olano, A. and Ramos, M. (1996): Protein changes in stored ultra-high temperature-treated milks studied by capillary electrophoresis and high-performance liquid chromatography. J. Agric. Food Chem., 44(12): 3955-3959.

Robitaille, G. (1995): Influence of kappa-casein and beta-lactoglobulin genetic variants on the heat stability of milk. J. Dairy Res., 62(4): 593-600.

Sendecor, G.W. and Cochran, W.G. (1989): Iowa's Statistical methods 7th ed Tate Univ. press.

Sinaga, H.; Bansal, N. and Bhandari, B. (2017): Effects of milk pH alteration on casein micelle size and gelation properties of milk. Int. J. Food Prop., 20 (1): 179-197.

Smith, M.C.; Madec, S.; Coton, E. and Hymery, N (2016): Natural co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. Toxins, 8(4): 94, 36p.

Stumpf, M.T.; Fischer, V.; McManus, C.M.; Kolling, G.J.; Zanela, M.B.; Santos, C.S.; Abreu, A.S. and Montagner, P. (2013): Severe feed restriction increases permeability of mammary gland cell tight junctions and reduces ethanol stability of milk. Animal, 7(7): 1137-1142.

Tsioulpas, A.; Lewis, M.J. and Grandison, A.S. (2007): Effect of minerals on casein micelle stability of cows' milk. J. Dairy Res., 74(2):167-73.

Walstra, P.; Wouters, J.T.M. and Geurts, T.J. (2005): Dairy Science and Technology. 2nd ed. (Food Science and Technology). CRC/Taylor & Francis, Boca Raton, FL.

Wang, Q.; Zhang, Y.; Zheng, N.; Guo, L.; Song, X.; Zhao, S. and Wang, J. (2019): Biological system responses of dairy cows to aflatoxin B1 exposure revealed with metabolomic changes in multiple biofluids. Toxins, 11(77): 22p.

Xiong, JL.; Wang, Y.M.; Nennich, T.D.; Li, Y. and Liu, J.X. (2015): Transfer of dietary aflatoxin B1 to milk aflatoxin M1 and effect of inclusion of adsorbent in the diet of dairy cows, J. Dairy Sci., 98 (4): 2545-2554.

Zanela, M.B.; Fischer, V.; Ribeiro, M.E.R.; Barbosa, R.S.; Marques, L.T.; Stumpf Jr, W. and Zanela, C. (2006): Unstable nonacid milk and milk composition of Jersey cows on feed restriction. Pesqui. Agropecu. Bras., 41(5):835-840.

Zanela, M.B.; Ribeiro, M.E.R.; Fischer, V.; Gomes, J.F. and Stumpf Jr, W. (2009): Occurrence of unstable non-acid milk in the Northwest of Rio Grande do Sul. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 61(4): 1009-1013.

العلاقات المتبادلة بين مكونات اللبن وخصائص البروتين والأنماط الجينية لجين الكابا كازين والثبات الكحولى في لبن الأبقار

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هدفت هذه الدراسة إلى الوقوف على بعض من الأسباب المتعددة التي قد ترتبط وتأثر على عدم الثبات الكحولى للبن بما في ذلك مكونات اللبن ، والملف التجزيئ لبروتين اللبن ، وتعدد أشكال جين الكابا كازين والأفلاتوكسين في كل من علف الحيوانات الحلابه (AFB1) والألبان (AFM1). تم تجميع عينات من لبن أبقار ودم كامل مجمع على مادة الإيديتا من ١٦٢ بقرة من بين ٤ مزارع أبقار هولشتاين والتي عانت من رفض تنكات الحليب الخام من قبل المصانع بسبب عدم إستقرار اللبن للإيثانول (تجبن اللبن). تم تقسيم عينات اللبن إلى مجموعتين حسب ثباتها للإيثانول. تم إخضاع جميع عينات اللبن لقياس كل من مكونات الحليب (البروتين ، الدهون ، اللاكتوز ، المواد الصلبة غير الدهنية ، المواد الصلبة الكلية والأملاح) ، الرقم الهيدروجيني ، عدد الخلايا الجسيمية والعد البكتيري الكلي. تم فحص ٣٠ عينة فقط من اللبن للكشف عن الأفلاتوكسين (AFM1) باستخدام كواشف الإليزا. أيضا تم جمع ٣ عينات مختلطة عشوائيه من كل مزرعة (١٢ عينة علف من كل المزارع) وتم إختبارها للكشف عن الأفلاتوكسين في العلف المستخدم فى هذه المزارع (AFB1) بإستخدام إختبار الإليزا. تم فحص عينات الدم للكشف عن الأنماط الجينية لجين الكابا كازين بإستخدام إختبار البلمره المتسلسل (PCR-RFLP). وقد تم تسجيل نسب ٧٢,٢٪ و ٢٧,٨٪ من عينات اللبن كحليب مستقر للإيثانول وغير مستقر على التوالي. أيضا كان هناك إنخفاض كبير في نسب كل من البروتين والدهون والمواد الصلبة الكلية ودرجة الحموضة للبن الغير المستقر مقابل اللبن المستقر للإيثانول مع زيادة ملحوظة في الأملاح وعدد الخلايا الجسيمية والعدد البكتيري الكلي. أيضا لوحظ فرق كبير في تركيز الأفلاتوكسينAFM1 في اللبن المستقر مقابل اللبن غير المستقر للإيثانول (٠,١٢٦ ± ٠,٠٢٥ مقابل ٤,٠٠٦ ± ٠,٢٦٤ جزء في تريليون). وقد تراوح تركيز الأفلاتوكسين AFB1 في عينات العلف للحيوانات الحلابه من ١٨,٥٨ إلى ٣٤,٥٨ جزء في البليون (متوسط ٢٦,١٤ ± ١,٢٨ جزء في البليون). علاوة على ذلك، لوحظ وجود فروق ذات دلالات إحصائية متغيرة في مستوى أجزاء البروتين المختلفة في اللبن وخاصة الكازين والذى أظهر أعلى إختلاف في عينات اللبن التي أظهرت عدم إستقرار للإيثانول مقارنة باللبن المستقر للإيثانول (٩,٩٦ ± ٠,٤٤ مقابل ١٧,٨٤ ± ٠,٥٢ على التوالي). وقد أظهرت الأنماط الجينيه لجين الكابا كازين أن ٥١ و ٨٧ و ٢٤ بقرة كانت من طرز وراثية AA و AB و BB على التوالى. أيضا أظهرت النتائج نسبًا أعلى بكثير للدهون ونسبة المواد الصلبة الكلية ومن ناحيه أخرى كان العدد البكتيري الكلى أقل بشكل ملحوظ في لبن الحيوانات ذات الأنماط الجينية BB مقابل كل من الأنماط الجينية AA و AB. وقد خلصت هذه الدراسه إلى أنه يمكن إعتبار المراقبة المستمرة ومتابعة معايير مكونات اللبن المختلفة وصحة الضرع التي يمثلها عدد الخلايا الجسيميه والعد البكتيري الكلى ومستوى الأفلاتوكسين في العلف الحيواني بمثابة خطوات أولى نحو إنتاج حليب ذو ثبات كحولى عالى.