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REGULAR ARTICLES

Milk traits of lactating cows submitted to feed restriction

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Abstract Data from five experiments with dairy cows where feed was restricted to 0, 40, and 50 % of the ad libitum amount, with 259 observations, were subjected to multivariate analyses to determine the effects of severity and duration of feed restriction on production, physical-chemical characteristics, ethanol stability, and somatic cell score of milk. A negative relationship was seen between the severity and duration of feed restriction with milk production, lactose content, titratable acidity, and milk stability to the ethanol test. The milk stability to the ethanol test, protein content, milk yield, and somatic cells score were the most important attributes retained by the discriminant analysis. Milk stability to the ethanol test, live weight, days in restriction, and pH were the most important characteristics explaining the variance within the different levels of feed restriction. Milk production and ethanol stability were significantly lower in both levels of feed restriction compared with the group fed ad libitum. When feed restriction was followed by refeeding, the difference observed in ethanol stability was the first discriminant variable, followed by the difference in unstable milk frequency and titratable acidity. Increments in the severity and duration of feed restriction negatively affect milk production and milk ethanol stability.

Keywords Ethanol stability · Milk production · Milk components · Nutrient limitation · Dairy cattle

Abbreviations

BCS	Body condition score
BW	Body weight
DIM	Days in milking
SCS	Somatic cell score
SCC	Somatic cell count
TS	Total solids of milk
UMF	Unstable milk frequency

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Introduction

Unfavorable environmental and seasonal conditions lead to shortage or limitation of food supply to livestock (Reiber et al. 2013). Water deficit in the hotter period of the year, inappropriate use of alternative forages for during the season of feedstuffs shortage, and inadequate management skills on farms are prevalent causes of feed restriction of dairy herds in tropical (Garg et al. 2013) and subtropical areas (Hills et al. 2015).

Feed restriction triggers fast mobilization of body tissues for maintenance as well as productive and reproductive activities of the animal (Schütz et al. 2013), causing metabolic and adaptive body changes. Frequently reduction in milk yield is seen, as well as decrease in the percentage of fat and protein of milk after the fifth day of restriction (Burke et al. 2010), an increase in SCC (Van Straten et al. 2009) and decrease in milk stability (Zanela et al. 2006; Barbosa et al. 2012). However, results varied between experiments and their comparison is difficult due to differences in the severity and duration of feeding restriction.

Multivariate analyses can be used to examine the effects of different magnitudes of feed restriction on milk production and milk components, as well as the stability of milk (Macciotta et al. 2012) as they allow for the recognition of the dependence between response variables of several experimental units while increasing the ability to interpret the data available which may not be possible in a univariate analysis.

The aim of this work was to investigate and evaluate variation in milk production as well as physical and chemical characteristics of milk from dairy cows submitted to different severities and durations of feed restriction.

Material and methods

Data from five experiments, conducted between years 2003 and 2013 which tested the effects of feed restriction on the milk characteristics, were used. All were carried out by researchers of the Federal University of Rio Grande do Sul, Brazil (UFRGS), Federal University of Pelotas, Brazil (UFPEL), Santa Catarina University State, Brazil (UDESC), and the CNPCT-EMBRAPA, Brazil. These studies were selected because they covered two feed restriction levels; cows were kept under similar management, climate, and conditions, and considered the same variables for all experiments. The restriction level was considered to be the amount of feed that was not provided as a percentage of that recommended by NRC (2001). For example, in a restriction level of 40 %, only 60 % of the diet recommended by NRC for this category and its production level were offered to the animals under restriction. The experimental data used were authorized by the Ethics Committees of each of the institutions involved. Table 1 presents information about each one of experiments utilized in this study.

These experiments were conducted between latitudes 27° 42′ S, 31° 45′ S, with Köppen climate classification Cfa, characterizing a subtropical zone (Alvares et al. 2013). Experiments 1, 3, and 4 were carried during warm season, while experiments 2 and 5 were conducted during the autumn and winter in the region, but during the procedure of the experiments, weather was not recorded. The original variables included in the multivariate analysis were severity and duration of the feed restriction, daily milk production, BW, BCS, milk pH, titratable acidity, ethanol stability, UMF, density, fat, protein, lactose and TS contents, SCS calculated as [$\log_2$ (SCC/100,000)+3]. Breed was classified numerically as 1 for Jersey and 2 for Holstein. The ethanol stability test was defined as the lowest concentration of ethanol in the alcoholic

Table 1 Characteristics of experiments conducted by UFRGS, UFPEL, UDESC, and CNPCT-EMBRAPA in working with feed restriction for lactating cows

Experiment	Animal characteristics	Days in experiment	Concentrate composition	Amount based in 100 % supply according NRC (cow/day)
1 (40 % restriction according NRC)	8 Jersey lactating cows, with BW 393±34.6 kg, BCS 3±0.5, daily milk production 13±3.3 L, and DIM 162±36.6 days	18 days (full and restriction)	Corn (60 %), soybean meal (36 %), wheat meal (1.7 %), dicalcium phosphate (2.3 %), and mineral premix	35 kg corn silage+6.1 kg of concentrate
2 (40 % restriction according NRC)	6 Jersey lactating cows with BW 382±15.4 kg , BCS 2.6±0.33, daily milk production 16.4±3.2 L, and DIM 48.3±30.4 days	30 days (full and restriction)	Sorghum grain (41.2 %), soybean hulls (47 %), soybean meal (10.3 %), dicalcium phosphate (1.5 %)	6.44 kg concentrate+4 kg alfalfa hay+oat and ryegrass pasture
3 (40 % restriction according NRC)	12 Holstein lactating cows, with BW 572.4±70.6 kg, BCS 2.7±0.3, daily milk production 18.9±5.4 L, and DIM 146.3±50.4 days	13 days as pre-experimental period and 4 days as experimental period	Soybean meal, ground corn, mineral salt	32 kg maize silage+4 kg tifton silage+4 kg soybean meal+3.4 kg ground corn
4 (50 % restriction according NRC)	12 Holstein lactating cows with BW 499±47.2 kg, BCS 3.02±0.29, daily milk production 19.35±4.10 L, and DIM 188±124 days	14 days (full and restriction)	Corn (67.6 %), barley (29 %), mineral premix (2 %), limestone (1 %), protein supplement (75 % soybean grain+25 % soybean meal)	Tifton (pasture+hay)+5 kg concentrate. Cows producing more to 18 L milk/day+2 kg protein supplement
5 (50 % restriction according NRC)	12 Jersey lactating cows with BW 372±33 kg, BCS 2.7±0.1, daily milk production 12.1±2.9 L, and DIM 145±39 days	7 days (full and restriction)	3.3 kg soybean, 2.6 kg corn, 240 g dicalcium phosphate, and 14 g $CaCO_3$	15 kg sugarcane silage+5.8 kg alfalfa hay+6.2 kg concentrate+0.16 kg mineral premix

solution capable of promoting coagulation of the milk, while the UMF was calculated as the percentage of milk samples coagulated after being mixed with equal amount of 76 % ethanol in alcoholic mixture relative to the total number of samples. These measures confirm the capacity of milk to support thermal treatment in industry.

All statistical procedures were performed using the statistical software SAS for Windows version 9.3 (SAS Institute Inc., 2002). Data was standardized with PROC STANDARD, with mean equal zero and standard deviation equal 1. The total number of observations from five experiments was 259, distributed between the control (0 % restriction diet), 40, and 50 % feed restriction.

Data standardized were analyzed for factor analysis (PROC FACTOR), arranged hierarchically according to feed restriction levels studied (PROC CLUSTER) and their arrangement analyzed in relation with the original variables by canonical analysis (PROC CANDISC). The original variables were ordered using the procedures PROC DISCRIM and PROC STEPDISC. The PROC VARCLUS determined the importance of the original variables in each restriction level. The comparison of the control (no restriction) with other levels of feeding restriction was conducted with a multivariate analysis (MANOVA), using PROC GLM, and levels of 40 and 50 % feed restriction were compared with group without restriction by Dunnett's test at 5 % significance. The evaluation of changes in the attributes of the dairy cows subjected to the sequence of feed restriction and subsequent refeeding (non-restricted) was performed by PROC CANDISC to describe the differences in the observed variables between each reversal period and by PROC STEPDISC to determine the discriminant variables in the process of restriction and refeeding of animals.

Results

Fifteen eigenvalue factors evaluated explained 100 % of the cumulative variance of the data. The first two main factors explained, respectively, 45 and 39 % of total variance. The first factor was closely related with milk fat, protein, and TS contents, while the second factor was more related with the severity and duration of feed restriction and UMF. Principal factor analysis showed (1) the strong and positive association between the severity and duration of the restriction with UMF; (2) the strong and positive association between fat, protein, and TS contents; and (3) the positive association between milk production, lactose content, ethanol stability, and titratable acidity (Fig. 1). Secondarily, an inverse relationship was found between the severity and duration of feed restriction with milk stability to the ethanol test, milk production, and lactose content. Considering the angle between the vectors (approximately 90°), severity and duration of feed restriction showed little relation with fat, protein, and TS contents, as well as with BW and BCS.

The canonical analysis confirms the effect of feed restriction on milk production and its physical-chemical characteristics. Daily milk production, titratable acidity, lactose content, and milk stability to the ethanol test were more related to the treatment without restriction, while pH, UMF, milk fat and protein content, and SCS were more closely associated with severity and duration of feeding restriction (Fig. 2). The key variables discriminating between the groups according to the severity of restriction were milk stability to the ethanol test, days in restriction, unstable milk frequency, and milk density (Table 2).

The original attributes that better explained the variance within feeding restriction levels were distinct according to

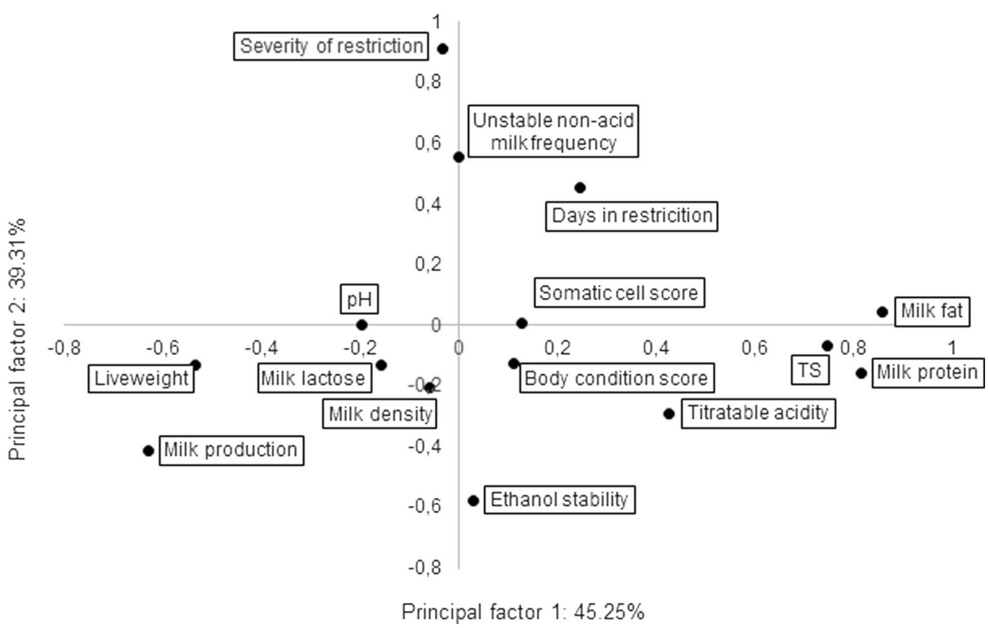

Fig. 1 Milk production, milk characteristics and stability of milk, severity and duration of the feed restriction designed in the orthogonal plane of the principal factors 1 and 2

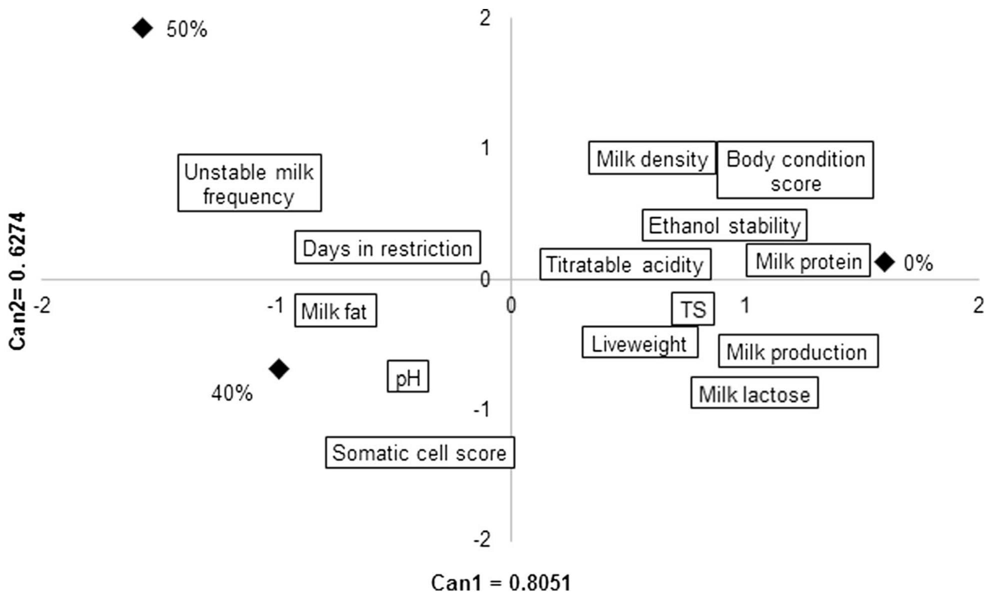

Fig. 2 Canonical means of productive attributes and physical-chemical characteristics of milk from cows subjected to levels of feed restriction 0, 40, and 50 %

the severity of restriction. The most important attributes to explain the variance within the groups without restriction were associated with titratable acidity and pH, but observations from the 40 % feed restriction group were linked with days in restriction and live weight while observations from the 50 % feed restriction group were mainly linked to ethanol stability (Table 3).

Compared to the animals fed without feed restriction, milk production and protein content were lower at the high level of restriction (50 %). The pH was greater in the 40 % restriction level, while the milk stability to the ethanol test was lower in both restriction levels (Table 4).

In the study of feeding-restriction-refeeding sequence, canonical correlation 1 shows that the major difference between the observations occurred in the application of the feeding-restriction-refeeding sequence in animals, regardless the level of restriction applied. Moreover, canonical correlation 2 shows the difference between levels of restriction applied to the animals. The largest differences in the measurements of the variables BW, BCS, and SCS were associated when the animals were subjected to the feeding-restriction sequence, whereas the largest observed difference in the other attributes were associated with the period in which the animals are subjected to restriction-refeeding (Fig. 3). The main attributes which showed significant discriminating power between both reversions sequences of restriction and refeeding were stability to the ethanol test and UMF (Table 5).

Table 2 Discriminant analysis of original variables for ordering of observations to levels 0, 40, and 50 % feed restriction

Original variable	Partial R^2	*P>F*	*P*>ASCC
Ethanol test	0.322	<0.0001	<0.0001
Days in restriction	0.303	<0.0001	<0.0001
Milk density	0.189	<0.0001	<0.0001
pH	0.149	<0.0001	<0.0001
UMF	0.139	<0.0001	<0.0001
Titratable acidity	0.114	<0.0001	<0.0001
Daily milk production	0.084	0.0002	<0.0001
Protein content	0.051	0.0014	<0.0001
Lactose content	0.069	0.111	<0.0001

Discussion

Feed restriction may reduce the supply of nutrients proportionally, but this depends on whether there was a reduction in the supply of the total diet or just part of it and it usually causes or exacerbates a negative energy balance. Lactating cows seek to minimize this effect by adopting strategies that involve (a) decrease of milk production and (b) increase mobilization of body reserves. Adoption depends on the genotype, stage of lactation, and magnitude of nutritional deficit (Gross et al. 2011; Bjerre-Harpøth et al. 2012).

In the present study, proportionality between concentrate: roughage and among nutrients was maintained between non-restricted and feed-restricted diets within trials. Besides that,

Table 3 Variance explained by the original variables within each level of feed restriction and control group

Treatment	Original variables	Proportion of variance explained by original variable (%)
0 % restriction	Titratable acidity, pH	77.5
40 % restriction	Days in restriction, BW	81.1
50 % restriction	Ethanol stability	86.8

Table 4 Means and standard deviations for the productive attributes and characteristics of the milk produced according to the severity of the restriction

	Restriction level			
	0 % (*n*=107)	40 % (*n*=118)	50 % (*n*=34)	*P*
Days of restriction	0	6.6±9.1	11.5±3.4	<0.0001
Breed[a]	1.6±0.5	1.8*±0.4	1.6±0.5	0.0105
Milk production (L/ cow/ day)	17.6±6.6	15.1*±4.6	10.4*±4.1	<0.0001
BW (kg)	465.7±85.9	457.4±50.9	440.3±76.3	0.184
BCS[b]	2.7±0.3	2.5*±0.2	2.6±0.5	0.0016
Density (g/L)	1030.2±0.9	1029.3*±0.3	1030.1±1.6	<0.0001
Fat (%)	4.1±0.9	4.2±0.8	4.2±0.7	0.478
Protein (%)	3.3±0.4	3.1*±0.4	3.2±0.4	0.0023
Lactose (%)	4.4±0.2	4.4±0.3	4.4±0.2	0.231
TS (%)	12.9±1.4	12.8±1.2	12.7±1.0	0.627
SCS[c]	2.5±2.3	3.8*±3.0	2.1±1.5	0.0003
pH	6.7±0.2	6.8*±0.1	6.7±0.1	<0.0001
Ethanol test (% ethanol)	75.3±3.5	68.6*±5.6	70.2*±3.9	<0.0001
Titratable acidity (°D)	16.6±1.8	15.1*±1.6	15.1*±1.8	<0.0001
UMF[d]	0.4±0.4	0.6*±0.2	0.9*±0.3	<0.0001

*Significantly different ($P<0.05$) of 0 % restriction group

[a] Breed: 1, Jersey; 2, Holstein

[b] Scale 1 to 5

[c] SCS: $\log_2$(CCS/100.000)+3

[d] 0, stable to ethanol test; 1, unstable to ethanol test

most of the cows were in the mid-lactation and producing moderate amounts of milk for tropical and subtropical conditions. Under these conditions, cows compensated for the reduced supply of nutrients principally by decreasing milk production without major alterations on BW and BCS. During mid-lactation milk yield and energy balance are more

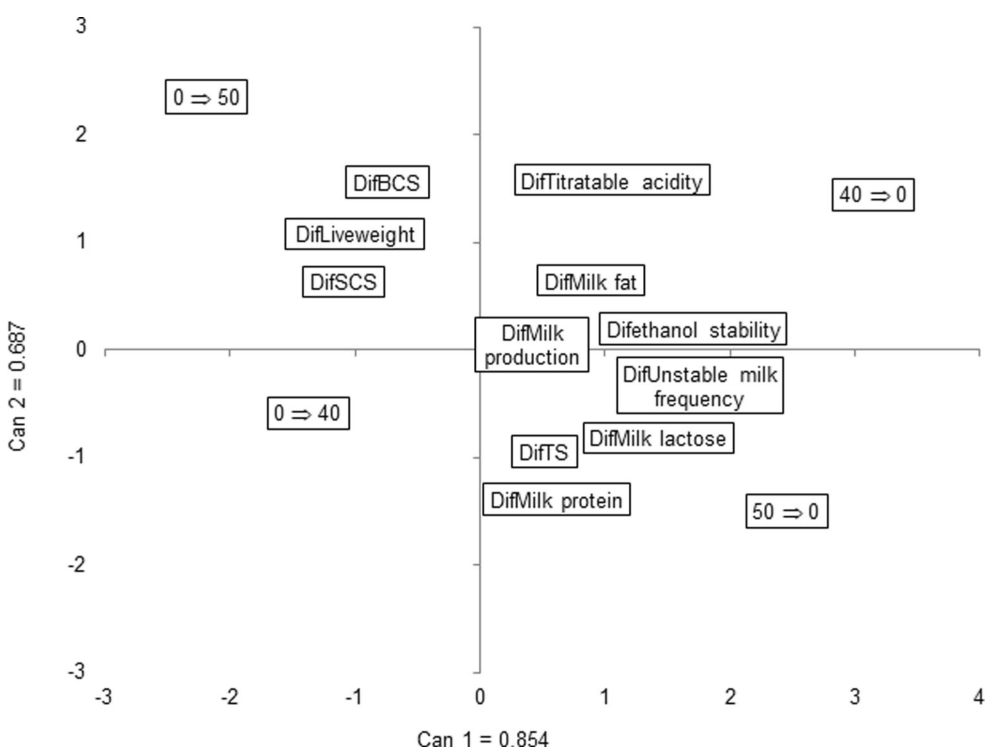

Fig. 3 Class mean of differences of productive attributes and physical-chemical characteristics of milk from cows subjected to feed restriction levels 40 and 50 %, considering the periods of restriction and refeeding

Table 5 Discriminant analysis of the differences among the variables in the sequence of restriction and refeeding to levels of feed restriction 0, 40, and 50 %

Variables	Partial R^2	*P>F*	*P*>ASCC
Difference in ethanol stability	0.285	<0.0001	<0.0001
Difference in UMF	0.242	0.0117	0.0001
Difference in titratable acidity	0.131	0.0991	0.0001
Difference in BW	0.099	0.152	0.0001
Difference in milk protein	0.035	0.1801	0.0001

related to DMI as cows can increase intake to face nutritional demands of milk production, while in the beginning of the lactation, they have to mobilize body reserves to counterbalance the extra nutrient demand for milk synthesis. Guinard-Flament et al. (2007) and Bjerre-Harpøth et al. (2012) also observed a reduction of milk production and a moderate increase of lipid mobilization of cows with feeding restriction.

The absence of significant differences in BW and very small differences in BCS as a result of feed restriction seems to be associated with modest average milk production and the lactation stage of the cows used in the experiment, compared with the cows used by Gross et al. (2011), where the animals started the experiment producing about 27 kg milk/day. In those studies, cows under feed restriction lose BW and BCS, unlike the data presented in the present study, where the relatively low production and quite advance lactation stage did not require an expressive mobilization of body tissues to face the nutritional shortage.

In the present work, both principal factor analysis and canonical analysis showed that duration and severity of the restriction were associated with lower milk production. These results are in agreement with those reported by Burke et al. (2010), who concluded that feed restriction for shorter periods but with high severity adversely affect milk production. In the same manner, Dessauge et al. (2011) also reported that restriction of nutrients leads to lower milk production and body condition score.

The negative association between lactose content and the severity and duration of restriction observed in the principal factor analysis as well as protein content changes observed for the 40 % feed restriction group are partially explained by the decrease of the synthesis of those components and by the differential magnitude of the reduction in the volume of milk and synthesis of milk components (Lacy-Hulbert et al. 1999; Burke et al. 2010; Bjerre-Harpøth et al. 2012).

However, we can almost discard the large SCS values as primarily related to lower milk lactose as they were observed just at the level of 40 % feed restriction. Lacy-Hulbert et al. (1999) and Van Straten et al. (2009) related feed restriction with increased SCC, which can be partially attributed to the higher rate of apoptosis observed by Dessauge et al. (2011), although Moyes et al. (2009) did not find differences in immune response between cows in mid-lactation submitted or not to energetic restriction of 40 % for 7 days after being challenged with injection of *Streptococcus uberis* in one of the mammary quarters.

Attributes as such acidity, ethanol stability, and UMF were highly associated with the severity and duration of feed restriction, which could be partially related to increased permeability of tight junctions between mammary epithelial cells, as demonstrated by Stumpf et al. (2013) who submitted cows to 50 % feed restriction and verified the largest influx of sodium by the paracellular route. This could also explain the inverse relationship between titratable acidity and the UMF (Figs. 1 and 2), in accordance with results of Marques et al. (2010), where a higher UMF in milk samples was related with titratable acidity less than 18 °D.

Feed restriction causes physiological and productive disorders in animals, and the refeeding procedure enables to the cows restore physiological conditions that prevailed in the period preceding feed deprivation (Xie et al. 2012). The data obtained for feeding-restriction-refeeding sequence are similar to found by Gross et al. (2011), who utilized a control group and another with 30 % feed restriction, observed statistical differences between groups but in refeeding period these statistical differences were null.

Conclusions

Feed restriction impacts negatively on milk production, concentration of milk components, mainly protein, and ethanol stability although are modulated by its severity and duration. Severe restrictions (50 %) even for relatively short periods (7 days) impair milk yield and composition. In addition to losses in milk yield and its components, there may be economic losses due to the reduction in protein content as well as rejection of milk due to the loss in stability.

Conflict of interest The authors declare that they have no conflict of interest.

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