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The effect of diet restriction on raw milk stability: a meta-analytical approach

Lisiane da Silveira Garcia, Arthur Fernandes Bettencourt, Júlia Fernandes Aires, Ines Andretta and Vivian Fischer

Animal Science Department, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Research Article

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Corresponding author:
Vivian Fischer; Email: vivian.fischer@ufrgs.br

Abstract

This research communication was designed to evaluate the effects of different levels of diet restriction on the composition and ethanol stability (MES) of raw bovine milk. This research was carried out using three electronic databases: Scopus, Pubmed and Web of Science. The main inclusion criteria were: (i) original research, (ii) use of alcohol (ethanol) test as a method to assess milk stability, (iii) measure different levels of feed restriction and (iv) allow access to the raw data of articles. Of the nine publications that addressed the subject filtered by the systematic review, seven fitted the selection criteria and were selected to perform the meta-analysis. Feed restriction (reduction of 20, 30, 40 and 50% of the dietary dry matter offered) decreased ($P < 0.01$) milk yield (−18%), ethanol stability (−5%), acidity (−4%), protein (−3%) and lactose (−2%) concentrations, but did not affect the values of pH, density, fat and total solids concentrations, nor somatic cell count. The correlation between milk yield and MES was low but positive and numerically higher in the control group compared with the restriction group. The milk of cows fed the control diet presented greater ethanol stability (76.5%) compared with milk of cows fed the restrictive diet (72.8%). This decrease by up to 4 percentage units due to restriction levels ranging from 20 to 50% of diet intake may cause limitations in milk processing at the dairy industry, increasing milk rejection.

Milk ethanol stability (MES) is influenced by several factors related to farm, environment, animals, feeding practices (Marques *et al*., 2010b; Martins *et al*., 2019) and animal health (Marques *et al*., 2011). It is already known that the raw milk stability decreases with reducing availability of pasture plus supplement (Fruscalso *et al*., 2013) or with TMR (Zanela *et al*., 2006; Gabbi *et al*., 2015) Decreased MES is also linked to a lower supply of nutrients (Stumpf *et al*., 2013) or an imbalance between nutrients such as energy and protein (Marques *et al*., 2010b; Gabbi *et al*., 2015; Schmidt, 2015), as well as with an increase in the fiber content of forages provided (Barchiesi *et al*., 2007). The effect of feed restriction on MES depends, among other factors, on its severity and duration (Gabbi *et al*., 2015). However, cows subjected to 40% reduction in TMR did not produce milk with lower stability (Barbosa *et al*., 2012), partially attributable to their management. The cows were semi-confined but could access cool-season high-quality pasture (*Avena strigosa* + *Lolium multiflorum*) during the day, and might have compensated for the lower TMR supply with higher ingestion of pasture. Nevertheless, milk yield was reduced by 13% and there was a trend of lower MES (2 percentage units) in 40% feed-restricted cows. Animal-related factors might have influenced these results, such as the lactation stage. Supplementation with alfalfa hay and concentrate did not improve MES of cows at late lactation, with more than 300 d in milk (DIM) (Marques *et al*., 2010a), or DIM > 210 d (Barbosa *et al*., 2012). On the other hand, milk stability is low (<72°GL) at the first two weeks *postpartum*, probably caused by the increase in Ca^{2+} (Tsioulpas *et al*., 2007).

The alcohol or ethanol test is a practical means of determining the susceptibility of bovine milk to coagulation by heat, especially when sophisticated quality testing is impractical (Guo *et al*., 1998). Thus, it is still used in several countries with relevant roles as players in the world milk production such as Brazil, Chile, Argentina, Uruguay, Russia, India and China. This research paper was designed to evaluate the effects of different levels of diet restriction on the composition and ethanol stability of raw bovine milk. It was hypothesized that dietary restriction modifies milk composition, resulting in lower MES.

Material and methods

Research methods to identify studies

We searched studies related to the ethanol stability of raw bovine milk in the three databases PubMed, Scopus and Web of Science. The search strategy was defined based on the main concepts in terms of PICo: population (P), intervention (I) and context (C). Subsequently, the search key was elaborated, and consisted of the following sequence: (milk) AND (UNAM OR unstable OR stability OR ethanol OR alcohol OR 'non acid' OR heat) AND ('feed restriction' OR 'thermal stress' OR 'heat stress' OR 'lactation stage' OR 'metabolic disease' OR 'metabolic disturb' OR 'digestive disease' OR acidosis OR mastitis OR agitation OR storage OR transport). The retrieved articles (n = 5,466) were evaluated by three researchers at different stages (more details in online Supplementary Fig. S1), namely the evaluation of titles, abstracts and full text. The following inclusion criteria were adopted: (i) use of the alcohol (or ethanol) test as a method for evaluating milk stability; (ii) evaluation of different levels of feed restriction based on dry matter and inclusion of a diet that met 100% of the animals' dietary needs (control diet); and (iii) access to raw data. No restrictions were imposed regarding the language or date of publication of the study.

After selection, nine articles were available for the systematic review (Supplementary Table S1). However, four articles could not be used in the meta-analysis (Supplementary Table S2). At this stage, an expert in the field was consulted, suggesting the inclusion of two other materials, one dissertation (Schmidt, 2015) and an unpublished experiment from 2006 (M B Zanela, Personal Communication). Thus, the present meta-analysis was carried out based on seven experiments (totaling 404 cows) that evaluated different levels of feed restriction and their relationship with MES (Supplementary Table S3).

Statistical analysis

Performance results were evaluated as raw data or expressed as proportion of the control. The proportional responses were called 'variation' (Δ) and may be interpreted as the 'constraint effect on each performance response'. Statistical analyses were performed using Minitab (Minitab for Windows, v. 20). Descriptive analysis and linear correlations (Pearson) were performed between the physical and chemical variables of milk (protein, fat, lactose, pH, acidity, alcohol (ethanol) stability, cryoscopy and somatic cell count, SCC). Means of milk components and MES of control and feed restricted groups were compared by variance analysis. The random study effect and fixed treatment effect were considered in all statistical models. In addition, the effects of the production system, genetic type, temperature and body condition score were tested for all responses, but maintained in the models only when considered as significant (defined for this purpose as *P-value* < 0.10). The interactions between the groups and other factors retained in the previous stage were tested, but no factor was maintained in the model, because no significant interaction was obtained. The residuals were tested for normality using the Ryan Joiner test for the final model. Interpretation of the treatment effect was performed at 5 and 10%. The regression adjustment between the variation (Δ, % in relation to the control group) of milk yield, quality and composition and the increase of feed restriction levels (%) was evaluated using linear and quadratic models. The number of observations per variable used in the models can be seen in Supplementary Table S4. Only the responses in which the effect of dietary restriction was significant ($P < 0.10$) were used in the analysis of variance.

Results

The correlation between milk yield and MES was positive (Fig. 1), higher in the control group ($r = 0.30$) compared with the feed restriction group ($r = 0.15$; $P < 0.05$). Also, MES showed a moderate correlation with lactose content only in the control group ($r = 0.39$; $P < 0.01$). In both groups, milk yield was negatively correlated ($P < 0.01$) with milk protein content.

Acidity was numerically correlated ($P < 0.10$) with MES, changing from negative (−0.09) in the control group to positive (0.14) in the feed restriction group which brings into question the biological significance. The correlations between acidity and protein contents were positive in both groups, with a greater value in the control group ($r = 0.39$; $P < 0.01$) and lesser in the restriction group ($r = 0.15$; $P < 0.05$). The correlation values between milk solids and protein ($r = 0.7$; $P < 0.01$) and fat content ($r = 0.9$; $P < 0.01$) were similar in control and feed restriction groups.

Overall, diet restriction (reductions of 20, 30, 40 and 50% of the supplied amount of diet, expressed as dry matter) reduced ($P < 0.01$) milk yield (−18%), MES (−5%), acidity (−4%), as well as concentrations of protein (−3%) and lactose (−2%), but did not affect pH values, density, fat concentration, total solids and SCC. Cows fed the control diet yielded milk with higher MES (76.5°GL) compared with feed restricted cows (72.8°GL: Table 1), corroborated by the values of the correlation coefficients. That is, when the animals receive the control diet, the correlations are positive, such as MES and milk yield or MES and lactose.

Milk characteristics negatively affected by feed restriction were milk yield (in 72% of comparisons), MES (77%), acidity (66%), protein content (74%) and lactose content (64%). The other characteristics of the milk were not influenced by the diet restriction. The regression analysis reveals that at each percentage unit of feed restriction imposed to animals there were significant reductions in milk yield (−0.4%), $y = -0.3994x$ ($R^2 = 0.36$; $P < 0.10$), in MES (−0.13%), $y = -0.1341x$ ($R^2 = 0.58$; $P < 0.10$), in milk acidity (−0.11%), $y = -0.1056x$ ($R^2 = 0.28$; $P < 0.10$), in protein content (−0.14%), $y = -0.1420x$ ($R^2 = 0.39$; $P < 0.10$) and in lactose content (−0.06%), $y = -0.0605x$ ($R^2 = 0.27$; $P < 0.10$). In more than 64% of observations, the diet restriction negatively affected all of these factors, ie production, MES, acidity, protein and lactose contents.

Discussion

This study confirmed the deleterious effect of feed restriction on milk functional characteristics such as MES, and established these reductions according to the magnitude of the feed restriction. The increase in each percentage unit of feed restriction reduced MES by 0.13%. Low milk stability may reduce or preclude some industrial product processing such as ultra-heat-treated milk. In addition, the deleterious impact of feed restriction on milk yield, acidity, and concentrations of protein and lactose were also confirmed, without changing pH, density, fat concentration, total solids and SCC values (Table 1).

The decrease in milk yield caused by feed restriction is due to the lower intake of nutrients, decreased energy intake and its lower uptake by the mammary gland, as the reduction of consumption reduces the blood flow to the mammary gland (Guinard-Flament *et al.*, 2007). The reduction in milk yield was observed in all studies that restricted feed supply (Zanela *et al.*, 2006; Barbosa *et al.*, 2012; Fruscalso *et al.*, 2013; Stumpf *et al.*, 2013; Schmidt, 2015). The relation between low MES and feed restriction may be explained by the stress caused by the restriction of feed supply and/or nutritional imbalance in a sudden way, altering animal behavior, e.g., increase in competition events and discomfort manifestations such as vocalizations, agonistic

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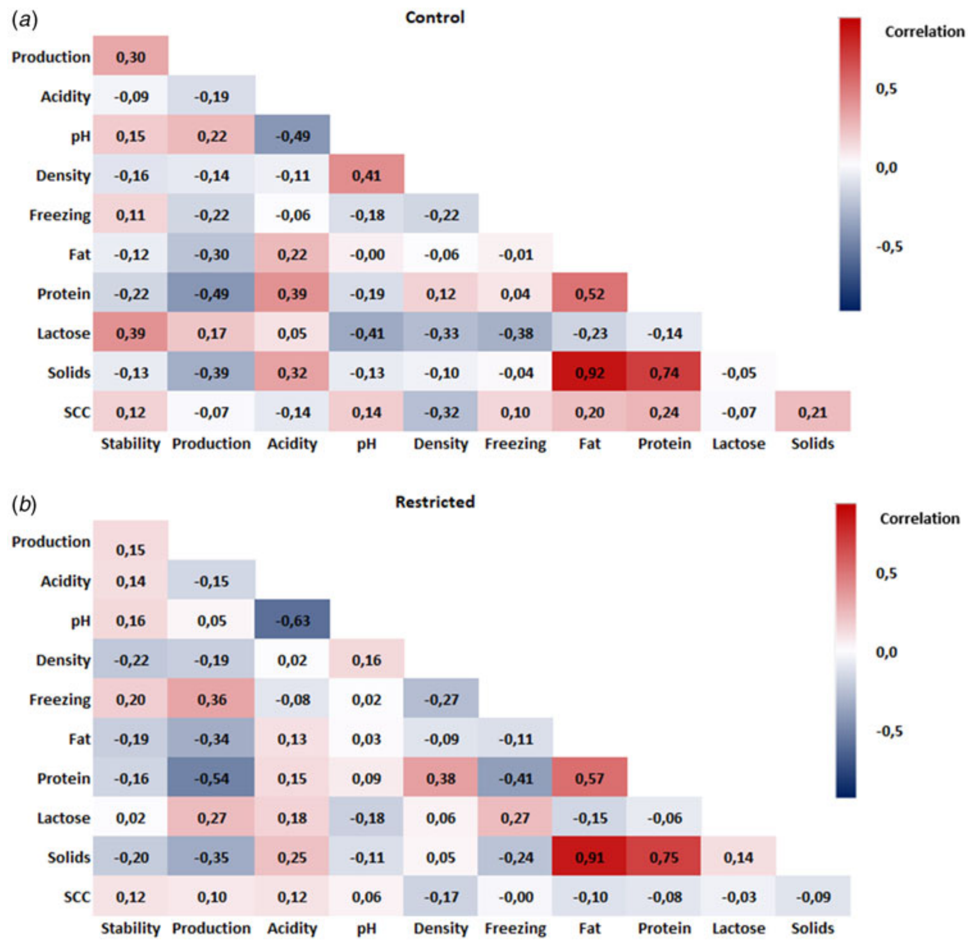

Figure 1. Correlations among milk yield, quality and composition in control (a) and feed restriction group (b).

behavior and stereotypies, resulting in increased cortisol secretion (Stumpf *et al.*, 2013, 2016). Moreover, these stress-induced behavioral and metabolic changes augmented plasma lactose concentration and sodium content in milk, while reducing lactose concentration in milk (Stumpf *et al.*, 2013), signaling an increased permeability of the tight junctions of mammary epithelial cells (Stelwagen *et al.*, 2000).

The decrease in protein and lactose concentrations due to feed restriction is related to the reduction of nutrient intake and thus, blood flow to the mammary glands, with lower absorption of nutrients, especially glucose decreasing synthesis of protein and lactose (Guinard-Flament *et al.*, 2007; Fagnani *et al.*, 2017). Moreover, the reduction in nutrient supply impairs synthesis of κ-casein, that is largely responsible for the stability of casein micelle (Gabbi *et al.*, 2018), as well of α-lactoalbumin, in turn linked to lactose synthesis and milk stability (Fagnani *et al.*, 2017). The reduction of the acidity as a function of the magnitude of the feed restriction may be related to the reduction of the protein concentration, which together with minerals such as phosphates and dissolved gasses contributes to the natural acidity of milk (Schmidt *et al.*, 1996). In the case of unbalanced diets with excess protein in relation to energy, the low stability observed (Marques *et al.*, 2010b; Schmidt, 2015) might be related to an excess of rumen degradable protein, which, in turn, is related to the lower glycosylation of κ-casein, reducing milk stability, as verified by Martins *et al.* (2019).

All studies included in this meta-analysis adapted the animals to a diet similar to the control treatment, from 7 to 17 d according to the study, before reducing suddenly the feed supply that, in turn, lasted between 7 and 14 d. This protocol highlighted the differences between the groups after the abrupt decrease in the diet supply. All studies were performed in experimentally controlled conditions. In commercial conditions, especially in developing countries, feed shortages may happen throughout the year in grazing and confinement systems due to environmental challenges faced by farmers, such as extreme weather conditions making access to pasture and feed transportation to farms difficult. In addition, farmers might sometimes change concentrate to forage proportions rather quickly to comply with variations in milk and feed prices, as there are far less government subsidies in developing countries.

Approximately 58% of the variation in MES was accounted for by the effect of feed restriction. However, only 27–39% of the variations in milk yield, acidity, protein and lactose concentrations are explained by feed restriction. Authors acknowledge the multifactorial nature of milk stability, reflecting variations in mineral composition (Tsioulpas *et al.*, 2007), acidity and caseins affected by feeding practices (Gabbi *et al.*, 2015, 2018) as well as animal health, breed (Vizzotto *et al.*, 2021), metabolic status (Marques *et al.*, 2011), lactation stage (Marques *et al.*, 2010a) and heat stress (Abreu *et al.*, 2020).

In conclusion, the hypothesis tested by the present study was accepted, since the present meta-analysis indicated, quantitatively,

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Table 1. Overall effects of feed restriction on milk yield, functional characteristics and composition

	Treatments						
Variables	Control	Restricted	Δ, %	RSE[a]	*P*-value[b]	Model[c]	Part.[d]
Milk yield, kg/day	14.51	11.85	−18.3	4.13	<0.001	G	12.1
Milk stability, ethanol, °GL	76.51	72.77	−4.9	4.15	<0.001	–	22.7
Acidity °D	16.69	16.01	−4.1	1.57	0.001	B	15.7
pH	6.72	6.74	0.3	0.17	0.151	–	0.3
Density, g/l	1030.1	1029.9	0.0	1.32	0.427	S, T	0.0
Freezing temperature, °C*	−0.543	−0.540	0.6	0.01	0.191	–	1.3
Fat content, g/100 ml	4.41	4.42	0.2	0.90	0.980	G	0.0
Protein content, g/100 ml	3.48	3.36	−3.4	0.35	0.005	G	11.8
Lactose content, g/100 ml	4.43	4.36	−1.6	0.25	0.016	G	12.2
Total solids, g/100 ml	13.20	13.06	−1.1	1.25	0.280	G	0.5
Somatic cell count, no × 1000 cells/ml	353.6	280.7	−20.6	350	0.243	–	0.6

[a]Residual standard deviation.
[b]*P*-value indicates the probability of feeding restriction effect.
[c]The random effect of studies was considered ($P < 0.10$) for all responses. The effects of production system (S), genetic type (G), temperature (T), and body condition score (B) were tested, but maintained in the models only when significant ($P < 0.10$). No significant interaction was found ($P > 0.10$).
[d]Partition of total variance attributed to feeding restriction effect.
*−0.512°C a −0.536°C – (Brasil, 2018).

that feed restriction reduces milk yield, lactose and protein concentrations, as well as MES. Milk ethanol stability decreased by up to four percentage units with dietary restrictions from 20 to 50%, which low stability may restrict the industrial use of this milk as well as causing increased milk rejection or devaluation by dairy industry.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000705

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