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Variation in bovine milk stability according to lactational stage and genetic group

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Research Article

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Abstract

We address the hypothesis that at early and late lactation milk presents low ethanol stability due to high acidity and ionic calcium values. Our aim was to evaluate the functional traits of milk (milk ethanol stability: MES, acidity and ionic calcium: iCa) according to lactation stage in different genetic groups. Raw milk samples were collected from Jersey ($n = 271$), Holstein ($n = 248$) and Jersey × Holstein crossbred cows ($n = 82$), raised on five commercial farms located in the state of Paraná, Brazil. Milk composition, somatic cell count (SCC), milk urea nitrogen (MUN), MES, pH, acidity and iCa were determined. Days in milk (DIM) were categorized into four classes: 1–60, 61–150, 151–305 and over 305 DIM. Data were submitted to analysis of variance. Fixed and random effects were incorporated into the model, in a repeated measures in time arrangement using the mixed models methodology. Significant interactions between DIM class and genetic groups were detected. The comparison between each combination of genetic group and DIM class showed that at the beginning of lactation, Holsteins produced milk with higher MES than Jersey and crossbreds. At 105–305 DIM Holstein milk presented higher MES than Jersey, while beyond 305 DIM Holstein milk showed higher MES than crossbred cows. At the beginning of lactation acidity was higher in Holstein milk and crossbreds compared with Jersey, while acidity was lower in Holstein milk compared with Jersey and crossbreds in the other lactation stages. Ionic calcium was highest after lactation peak for Holstein, but did not vary between lactation stages for Jersey and crossbreds. Functional characteristics of bovine raw milk such as MES, iCa and acidity varied between lactation stages in a distinct manner according to genetic groups. Early and end lactation stages are challenging in terms of low stability, especially for Jersey and crossbreds.

Milk ethanol stability (MES) is a functional characteristic of milk still measured in several countries such as Brazil, Uruguay and Argentina to indicate the adequacy of raw milk for thermal processing (Fischer *et al.*, 2012). MES is the primary test at on-farm level, before loading milk for transportation (Chavez *et al.*, 2004). Briefly, it is performed mixing an equal volume of milk sample from the bulk tank and ethanol solution, stirring and visual inspection for clots appearance. If there is clot formation, the milk is considered to be unstable (Brasil, 2018). Previously, the main reason associated with low MES was the high acquired acidity (pH <6.4 or acidity higher than 0.18% lactic acid), but recently low stability in milk showing acidity within the normal range values (0.14 to 0.18% lactic acid) has become more prevalent (Machado *et al.*, 2017; Martins *et al.*, 2024).

In the ultra-high temperature (UHT) process, milk is subject to temperatures above 135°C for a few seconds giving a product with a shelf-life of several months. In countries where UHT is the main form of fluid milk processing, thermal stability is of paramount importance to avoid fouling in equipment during processing or sedimentation and/or gelation during storage (de Souza *et al.*, 2023). Casein stability may be estimated using the ethanol test (Horne, 2016). Therefore, to endure the UHT process, milk should present MES of at least 76% (Shew, 1981).

Low milk stability is associated with protein compositional changes such as the proportions of kappa-casein, β-lactoglobulin and α-lactalbumin (Barbosa *et al.*, 2012; Fagnani *et al.*, 2018; Pinheiro *et al.*, 2022), high acidity or low pH (Chavez *et al.*, 2004) as well as variations in the concentration of mineral salts (Tsioulpas *et al.*, 2007*b*; Horne, 2016; Akkerman *et al.*, 2019; Pinheiro *et al.*, 2022). These arise from inappropriate physiological and metabolic conditions (Marques *et al.*, 2010, 2011; Martins *et al.*, 2015), heat stress (Abreu *et al.*, 2020), feed restriction (Fruscalso *et al.*, 2013; Stumpf *et al.*, 2013; Gabbi *et al.*, 2016) and nutritional imbalances (Marques *et al.*, 2010; Martins *et al.*, 2015; Gabbi *et al.*, 2016).

Moreover, low stability might be associated with the lactation stage, albeit there are few reports about evolution of milk stability during lactation (Tsioulpas *et al.*, 2007*a*). Milk stability is low at the beginning of lactation, especially during the first 10 d after calving, increasing progressively afterward (Tsioulpas *et al.*, 2007*a*; Vizzotto *et al.*, 2021). In addition, MES is low at the end of lactation, especially in extended lactation (Marques *et al.*, 2010). In early lactation, dairy cows are frequently challenged by negative energy balance (NEB) because the nutrient demand for milk synthesis is not fulfilled by diet intake. Excessive NEB can lead to metabolic disturbances and consequently decreased MES (Marques *et al.*, 2011). Also, subclinical ketosis and hypocalcemia are common in fresh cows (Santos *et al.*, 2019). Milk with low stability showed lower total calcium and phosphates compared to milk with high stability (Fagnani *et al.*, 2014). Moreover, negative relations between MES and ionic calcium have been reported (Chavez *et al.*, 2004; Lewis, 2011).

Relations between milk acidity and MES are scarcely reported, but usually they are negatively associated, especially when linked to high bacterial count (TBC: Chavez *et al.*, 2004; Machado *et al.*, 2017). However, milk acidity is associated with concentrations of natural milk components such as phosphates, citrates, caseins and albumin that may be influenced by factors such as breed, lactation stage, period of the year, nutrition and animal feeding (González, 2001; Fox *et al.*, 2015).

The main difficulty in comparing milk stability between genetic groups is to keep the animals under similar conditions of management, environment and nutrition, otherwise it generates a large variability of covariates that may affect the results. In addition, most studies have evaluated milk stability in Holstein cows (Tsioulpas *et al.*, 2007*a*; Fischer *et al.*, 2012; Horne, 2016). Exceptions include Jerseys (Heisler *et al.*, 2017; Vizzotto *et al.*, 2021) and Holstein and Holstein × Gyr crossbreds (Botaro *et al.*, 2007; Vizzotto *et al.*, 2021). Moreover, Botaro *et al.* (2007, 2009) reported that Holstein cows showed higher milk stability than Girolando cows.

Our hypotheses are, firstly, that cows at early and late lactation show low MES due to high acidity and iCa values, and secondly, these variations occur irrespective of genetic group. Thus, our aim was to evaluate the functional traits of milk (MES, acidity, iCa) according to lactation stage in different genetic groups.

Materials and methods

The experimental procedures were approved by the Research Committee of the Federal University of Rio Grande do Sul (no 41594).

Animals and general management

Raw milk samples of individual cows were collected at the morning milking, from August 2021 to August 2022, totaling 588 milk samples: Jersey ($n = 271$), Holstein ($n = 248$) and Jersey × Holstein crossbred ($n = 82$). Each month, the same person collected milk samples from the available cows in each farm. Cows were housed in five commercial farms located at the state of Paraná, Brazil. Description of farms, herd composition and animals' characteristics as well as meteorological conditions during the study are shown in the Supplementary material (Supplementary Tables S1 and S2).

On each sampling day, the following information about the animals were recorded: genetic group, days in milk (DIM), parity. Also, body weight (BW) was estimated using heart girth tape, and body condition score (BCS) was assigned by the same evaluator on a scale of 1 to 5 (Edmonson *et al.*, 1989). Nutritional, hygiene and milking management practices as well as heat stress mitigation tools were assessed based on farmer deposition and observation of daily routine during the technical visit, and each management was scored as adequate or inadequate, as follows: Nutritional adequacy = adequate farms were considered to be those with specialized technical assistance, balanced diets, e.g. TMR, adequate feed stocks, no feed deficit. Nutritional inadequacy = farms without balanced diets, without specialized technical assistance, semi-confinement diets without adequate pasture management, with a shortage of pasture in times of scarcity. Heat stress mitigation measures: adequate = use of shading and/or fan + sprinklers. Inadequate = no use or scarce during the year. Milking practices: adequate = adoption of most of the practices of cleaning of the udder, adequate vacuum level, pre and post dipping, cleaning of the equipment and parlor. Inadequate = absence of 2 or more items.

The temperature and humidity data were collected from the environmental weather station closest to each farm. The temperature and humidity index (THI) was calculated using the equation $THI = (1.8 \times T + 32) - [(0.55–0.0055 \times RH) \times (1.8 \times T - 26.8)]$ (NRC, 1971), where T = air temperature and RH = relative humidity.

Milk characteristics

Milk samples were collected from individual cows at the morning milking using whole milking sampler units. Samples were divided between two 40-ml plastic flasks with lid, one with conservative (Bronopol®) and other without conservative, all kept refrigerated. Flasks with conservative were shipped the day after their collection to the laboratory of the Paraná Association of Holstein Cattle Breeders for milk composition and somatic cells count (SCC) determination. The concentrations of fat, protein, casein, lactose, defatted dry extract, total solids extract (TS) and urea in milk (MUN) were determined by Fourier-transform infrared spectrophotometry (Bentley FTS, Bentley Instruments, Chaska, MN). SCC was determined by flow cytometry using Somacount 300 equipment (Bentley Instruments, Chaska, Minnesota, USA), following recommendations of the International Dairy Federation (2000).

Flasks without conservative were cooled at 4°C for 12 h without lid, and subsequently analyzed for pH and ethanol stability (MES). The pH was determined by potentiometry and MES was determined by mixing 2 ml of milk and 2 ml of alcoholic solutions with ethanol concentration of 68, 70, 72, 74, 76, 78, 80, 82, 84 and 86% v/v in a Petri dish (Tronco *et al.*, 2013). The results were expressed as the minimum concentration of ethanol in the alcoholic solution that induced visually detected milk coagulation.

The pH and acidity determination were performed by potentiometry and titration, respectively (Vidal and Saran Netto, 2018). Acidity was expressed as g of lactic acid per 100 ml of milk. Ionic calcium was determined by ion-selective potentiometry (Thermo Scientific Orion® potentiometer, Dual Star model) according to the methodology described by Ribeiro *et al.* (2010). The potentiometer had a combined reference electrode and a selective electrode for calcium ions. The iCa concentration values were expressed in mg/L.

Statistical analysis

Based on DIM, all observations were classified into four lactation stages, namely early, post-peak, late and extended lactation. In

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detail, these were 1–60 DIM ($n = 85$), 61–150 DIM ($n = 122$), 151–305 DIM ($n = 212$) and over 305 DIM ($n = 169$). The research was performed as a repeated measures observational study and the data were submitted to analysis of variance. Fixed effects and random effects were incorporated into the model, under the mixed model methodology. The variables genetic group ($n = 3$: Holstein, Jersey and their crossbreds) and DIM class ($n = 4$) were included as fixed effects. Parity was included in the model as covariate. Farm ($n = 5$) and season of the year ($n = 4$) were included as random effects and the day of evaluation, in each farm, was allocated as repeated measures in time, according to the model:

$$Y_{ijkl} = \mu + GG_i + DC_j + (GG^*DC)_{ij} + F_k + S_l + (F^*D)_{jm} + (P_n) + \varepsilon_{ijklm}$$

where: Y_{ijk} is the observation related to the i-th genetic group effect (GG_i), in the j-th DIM_class (DC_j) and their interaction. Parity (P_n) was included as covariate. Farm (f_k) and season (S_l) were considered as random effects, and (F^*D) as the day of evaluation on each farm was considered as repeated in time.

The adjustment of the random effects and the choice of the mathematical model were made by the corrected Akaike information criterion (AICc). For this purpose, the Glimmix procedure (SAS, 2013) was used, choosing the best distribution (Supplementary Table S3) and model fit, based on the AICc. Other random effects were tested, and their inclusion or exclusion from the model was based on the best fit, based on the criteria mentioned above. SAS OnDemand was used for statistical analysis.

Results

The overall average (±SD) number of days in milk per lactation stage class were 28 ± 1.82, 104 ± 2.87, 234 ± 3.60 and 428 ± 11.70 d for the different lactation stages. Significant ($P < 0.05$) interactions between lactation stage and genetic groups were detected for MES, acidity, iCa, fat, MUN, TS and SCC (Table 1).

MES did not vary between lactation stages in Holsteins. In Jerseys, the lowest and highest MES values were detected at early and post-peak lactation, respectively, while in the crossbreds, MES was lowest at the beginning and end of lactation (after 305 DIM) and highest after lactation peak. The comparison between each combination of genetic group and DIM class showed that at beginning of lactation, Holsteins presented higher stability than Jerseys and crossbreds. Also, during late lactation, Holsteins presented higher MES than Jerseys, while after 305 DIM Holsteins showed higher MES than crossbred cows.

At the beginning of lactation, acidity was highest in Holstein, not varying afterward. Conversely, in Jerseys, acidity was lowest at the beginning of lactation and highest during late and extended lactation. On the contrary, acidity did not significantly vary between lactation stages for crossbreds. Comparing genetic groups within lactation stage, at the beginning of lactation acidity was higher in Holsteins and crossbreds than in Jerseys, while acidity was lower in Holsteins compared with Jerseys and crossbreds in the other lactation stages.

Ionic calcium was highest after lactation peak for Holsteins, but did not vary between lactation stages for Jerseys and crossbreds.

Fat content did not vary between lactation stages in Holsteins, while in Jersey cows, the lowest and highest values for fat were

Table 1. Mean and standard error of the mean values for milk ethanol stability (MES), acidity, ionic calcium (iCa), concentration of fat, milk urea nitrogen (MUN) and somatic cells count (SCC) according to interaction between lactation stage (DIM class) and genetic group

	DIM	MES (°GL)	Acidity (g of lactic acid/100 ml)	iCa (mg/l)	Fat (g/100 g)	MUN (mg/dl)	Total solids (g/100 g)	SCC (×1000) cells/ml
Holstein	0–60 d	79.48 ± 1.90^{aA}	0.02 ± 0.01^{aA}	151.81 ± 51.62^{abA}	3.85 ± 0.20^{aB}	16.85 ± 1.91^{aA}	12.62 ± 0.23^{aA}	361 ± 2.7^{cC}
	61–150 d	78.91 ± 1.94^{aA}	0.18 ± 0.01^{bB}	180.16 ± 61.68^{aA}	4.04 ± 0.21^{aA}	15.95 ± 1.91^{aAB}	12.59 ± 0.22^{aA}	438 ± 3.3^{bC}
	151–305 d	79.90 ± 1.76^{aA}	0.19 ± 0.01^{bB}	140.47 ± 47.54^{bA}	3.91 ± 0.17^{aB}	14.04 ± 1.83^{abB}	12.54 ± 0.19^{aB}	523 ± 2.8^{aB}
	>305 d	79.04 ± 189^{aA}	0.19 ± 0.01^{bB}	134.61 ± 45.60^{bB}	3.71 ± 0.18^{aB}	13.64 ± 1.87^{bB}	12.53 ± 0.21^{aB}	188 ± 1.8^{dB}
Jersey	0–60 d	74.07 ± 218^{bB}	0.18 ± 0.01^{bB}	155.47 ± 54.01^{aA}	3.95 ± 0.27^{bB}	16.78 ± 2.22^{aA}	12.58 ± 0.31^{bA}	385 ± 4.5^{dB}
	261–150 d	78.82 ± 1.81^{aA}	0.20 ± 0.01^{abA}	137.13 ± 46.86^{aB}	4.19 ± 0.20^{abA}	17.64 ± 1.84^{aA}	13.06 ± 0.21^{bA}	656 ± 3.5^{aB}
	151–305 d	76.77 ± 1.76^{abB}	0.20 ± 0.01^{aA}	146.10 ± 49.52^{aA}	4.01 ± 0.19^{aA}	17.64 ± 1.84^{aA}	13.47 ± 019^{aA}	577 ± 2.5^{bA}
	>305 d	77.26 ± 1.75^{abA}	0.20 ± 0.01^{aA}	144.92 ± 49.13^{aB}	4.57 ± 0.21^{aA}	16.40 ± 1.86^{aA}	13.60 ± 0.22^{aA}	417 ± 2.6^{cA}
Jersey × Holstein crossbreds	0–60 d	74.01 ± 2.76^{bB}	0.22 ± 0.02^{aA}	158.80 ± 58.64^{aA}	3.76 ± 0.32^{abB}	15.57 ± 2.52^{aA}	12.58 ± 0.38^{abA}	775 ± 7.6^{aA}
	61–150 d	82.70 ± 2.41^{aA}	0.20 ± 0.02^{aA}	142.43 ± 50.42^{aB}	3.70 ± 0.25^{bA}	13.30 ± 2.17^{aA}	12.46 ± 0.29^{bA}	790 ± 6.4^{aA}
	151–305 d	77.28 ± 1.99^{bAB}	0.20 ± 0.02^{aA}	141.21 ± 48.58^{aA}	4.81 ± 0.24^{aA}	16.20 ± 1.97^{aA}	13.30 ± 0.26^{aA}	248 ± 2.8^{bC}
	>305 d	72.34 ± 2.58^{bB}	0.19 ± 0.02^{aAB}	173.33 ± 61.80^{aA}	3.49 ± 0.32^{bB}	16.08 ± 2.26^{aAB}	12.38 ± 0.41^{bB}	77 ± 3.6^{cC}
P-value interaction		0.0195	0.0196	0.0437	0.0421	0.0292	0.0185	0.0001
P-value parity		0.0033	0.0310	0.5848	0.0256	0.0505	0.0001	0.0001

^{a,b,c} means in the same column followed by the different letters within genetic group differ significantly ($P < 0.05$).
^{A,B,C} in the same column followed by the different letters within the same DIM class differ significantly ($P < 0.05$).

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observed at the beginning and end of lactation (after 305 DIM), respectively. On the other hand, in crossbreds, the lowest fat values were observed at post-peak of lactation and during extended lactation (after 305 DIM), while the highest values were detected during late lactation. At the first half of lactation, there were no differences between genetic groups. In the second half of lactation, Holsteins presented lower values than Jerseys and crossbreds.

Milk urea nitrogen values in Holsteins were lowest at late and extended lactation and highest during the first half of lactation, while MUN did not vary between lactation stages in Jerseys or crossbreds.

Total solids (TS) did not vary between lactation stages in Holsteins. In Jerseys, TS presented lowest values at the beginning of lactation compared to other stages. Crossbreds presented their lowest TS values at the post-peak and extended lactation stages, whilst the highest values were detected during late lactation.

Somatic cells presented lowest and highest values in extended and late lactation, respectively, in Holsteins, while in Jerseys these same differences were seen in early and post-peak lactation. Crossbreds presented lowest values extended lactation and highest values in early and post-peak.

There were significant ($P < 0.05$) differences for protein and casein concentrations between genetic groups, while lactose and pH did not differ between genetic groups (Table 2). Jersey and crossbreds showed higher overall milk protein and casein concentrations than Holstein.

There were significant ($P < 0.05$) differences for protein, casein and lactose between lactation stages, while pH did not vary (Table 2). During the first half of lactation protein and casein presented lower values than in the second half. Lactose presented the highest and lowest values at early and late lactation, respectively.

Discussion

It is known that concentration of milk components (protein, casein, fat) vary along lactation and between genetic groups (Fox *et al.*, 2015). It is far less evident how other characteristics such as MES, acidity, pH and iCa vary as lactation progresses and between genetic groups. These variables influence the functionality of milk and dairy products, as they affect their thermal processing, for example, increasing the presence of sediments and gels in the products during and after the application of heat in the UHT processes and drying of the milk for the manufacture of milk powder, and may affect the yield and sensory characteristics of dairy products such as cheeses and yogurts (Deeth and Lewis, 2017). MES is considered a good estimator of the thermal stability of milk, including for the UHT process (Chen *et al.*, 2012), showing sensitivity but no specificity (Deeth and Lewis, 2017). The main contribution of the present study is to provide evidence that functional characteristics of bovine raw milk such as MES, acidity, pH and iCa vary with lactation stage, but in different ways according to the genetic group.

In our study, the low values of MES at the beginning of lactation were coincident with numerical (albeit not significant) higher values for iCa for Jersey and crossbreds, and higher numeric values of acidity only for crossbreds. Previous studies showed that MES is low in the first 10–15 d after calving in Holstein cows (Tsioulpas *et al.*, 2007*a*) and Jersey cows (Heisler *et al.*, 2017). Besides, MES was negatively correlated with the concentrations of protein as well with iCa and calcium to inorganic phosphorus ratio (Chavez *et al.*, 2004; Li *et al.*, 2019).

Moreover, the low values of MES at the end of lactation were coincident with high acidity values in the milk of Jersey cows but not in crossbreds. On the other hand, low MES was coincident with numerically high iCa in crossbreds. Previously, as lactation progressed MES was reduced, but in a highly variable way (Marques *et al.*, 2010), and the cut point is still not reported, nor are any possible mechanisms.

The low values for MES at the beginning (Tsioulpas *et al.*, 2007*a*; Heisler *et al.*, 2017) and end of lactation (Marques *et al.*, 2010) have been related to changes in the concentrations of mineral salts (Tsioulpas *et al.*, 2007*b*; Horne, 2016), in turn resulting from differences in genetics (Botaro *et al.*, 2009; Fagnani *et al.*, 2018) and casein composition (Barbosa *et al.*, 2012; Martins *et al.*, 2019). In addition to the variations in MES between genetic groups at the different lactation stages, the association between MES and other functional characteristics also varied. For instance, the comparisons between genetic groups at each lactation stage evidenced the highest MES in Holsteins compared with Jerseys and crossbred cows at the beginning of lactation. Against our expectations, in early lactation (1–60 DIM), there were no

Table 2. Mean and standard error of the mean values for pH and milk concentration of protein, lactose and casein according to classes of days in milk (DIM_class) and genetic group

DIM	0–60 d	61–150 d	151–305 d	>305 d	*P*-value
Variables					
pH	6.79 ± 0.06^{a}	6.80 ± 0.05^{a}	6.79 ± 0.05^{a}	6.81 ± 0.06^{a}	0.6415
Protein (g/100 g)	3.27 ± 0.08^{b}	3.33 ± 0.07^{b}	3.53 ± 0.06^{a}	3.59 ± 0.07^{a}	0.0001
Lactose (g/100 g)	4.55 ± 0.08^{a}	4.42 ± 0.07^{ab}	4.30 ± 0.06^{c}	4.31 ± 0.07^{c}	0.0001
Casein (g/100 g)	2.66 ± 0.10^{a}	2.69 ± 0.07^{a}	2.89 ± 0.06^{b}	2.85 ± 0.07^{b}	0.0035
Genetic group	Holstein	Jersey	Jersey × Holstein	–	*P*-value
Variables					
pH	6.78 ± 0.06^{a}	6.81 ± 0.05^{a}	6.80 ± 0.06^{a}	–	0.3089
Protein (g/100 g)	3.30 ± 0.06^{b}	3.51 ± 0.07^{a}	3.48 ± 0.07^{a}	–	0.0001
Lactose (g/100 g)	4.43 ± 0.07^{a}	4.38 ± 0.07^{a}	4.40 ± 0.08^{a}	–	0.5708
Casein (g/100 g)	2.66 ± 0.06^{b}	2.80 ± 0.06^{a}	2.84 ± 0.09^{a}	–	0.0276

^{a,b,c} means in the same row followed by the different letter within lactation stage or within genetic group are statistically different ($P < 0.05$).

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differences in acidity (between Holsteins and crossbreds) and iCa between genetic groups to explain the distinct values of MES. We acknowledge that it would be important to have determined the concentration of other minerals, e.g., phosphates and citrates as well as caseins, but we do not have these data, which is a limitation of the study.

At the second lactation stage (post-peak, 61–150 DIM), the tendency of higher MES observed in crossbreds compared with Holsteins was coincident with higher acidity. Conversely, the higher values of MES during late lactation (151–305 DIM) in Holsteins compared with Jerseys and crossbreds were coincident with lower values of acidity. Martins *et al.* (2024) also observed a weak positive correlation between MES and acidity, and attributed this to the high concentrations of protein and phosphates (although they were not determined), since the TBC was low (81.7% of the samples presented TBC values lower than 100 000 CFU/ml of milk). Finally, at the extended lactation stage (>305 DIM), higher values of MES in Holsteins were coincident with lower iCa concentration compared with crossbreds. Previously, low MES was attributed to the high concentration of iCa and acidity in Holstein cows (Chavez *et al.*, 2004; Tsioulpas *et al.*, 2007*a*).

The higher values of fat, protein and casein concentrations in the second half of lactation are probably related to the reduction in milk yield after lactation peak, concentrating the milk components (Kappes *et al.*, 2020). The inverse pattern shown by lactose concentration is partially related to its role as the main osmoregulator and thus, presents positive correlation with milk yield (Fox *et al.*, 2015). Moreover, Alessio *et al.* (2016) reported that lactose content is influenced by SCC and parity, with seasonal variation, but is not related to breed, milk production, milk fat or protein content. Recently, Alessio *et al.* (2021) reported that variation in lactose concentration was not related to variations in protein and fat contents, but it was negatively related with SCC and TBC.

It is well known that Jersey cows produce milk with higher concentration of fat and protein than Holstein cows (Sanjayaranj *et al.*, 2022). The relationship between milk composition and MES is not yet fully understood, but increased sodium and chloride and decreased lactose content in milk are related to low stability (Chavez *et al.*, 2004; Tsioulpas *et al.*, 2007*a*; Fagnani *et al.*, 2018).

The reduction in MUN during the second half of lactation in Holsteins might be related to the low protein concentration in TMR usually fed to cows at the end of lactation (NRC, 2001). The lower MUN values observed for Holsteins compared with Jerseys and crossbreds at post-peak and late lactation might also be related to diet formulation by farms. Farms in the present study had mixed herd breeds, and in 4 out 5 of them, Holstein was the dominant genetic group. Thus, we hypothesize that the diets in these herds were formulated according to the milk yield and composition of the Holstein. Consequently, Holsteins would have a greater nitrogen utilization efficiency, which could lead to lower MUN concentrations. Moreover, Doska *et al.* (2012) performed a survey in 96 herds at Paraná State (south region of Brazil) and reported that Holstein cows showed lower MUN adjusted means than crossbred and Jersey cows. Also, these authors verified that MUN concentrations peaked between 151 and 180 d in milk, attributing this to the higher consumption of dry matter and consequently of crude protein during this stage of lactation.

The highest acidity of early lactation milk in Holstein cows may be related to the transition milk, in agreement with the results reported by Tsioulpas *et al.* (2007*a*). Natural milk acidity is related to phosphates, citrates, caseins, albumin and carbon dioxide (Veloso, 1998; Fox *et al.*, 2015), contributing with 0.14 to 0.18 g of lactic acid/100 ml of milk. However, casein variation does not explain acidity values, as they drop post-peak and were similar between other DIM stages. Therefore we might speculate that other components such as albumins, phosphates and citrates might have affected milk acidity. The reason why acidity increased as lactation progressed in Jersey cows are not easily explained.

The higher acidity values observed in Jersey cows (except in early lactation) and crossbreds compared with Holsteins is attributed to higher crude protein and casein concentrations (Neves, 2021; Ormston *et al.*, 2022). Overall, for Jerseys we can accept the hypothesis that cows at early and late lactation show low MES due to high acidity and iCa values, whilst we could not find evidence of a clear relation between MES and acidity and iCa for Holsteins and crossbreds.

In conclusion, functional characteristics of bovine raw milk such as MES, iCa and acidity varied between lactation stages in a distinct manner according to genetic groups. Early and end lactation stages are challenging in terms of low stability, especially for Jerseys and crossbreds, and also in terms of high acidity, although there is no consistency between genetic groups nor relation with total bacterial count. Ionic calcium varied between lactation stages only for Holsteins, and showed no clear pattern or consistency with ethanol stability and acidity.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000372.

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