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Phenotypic characterisation of milk technological traits, protein fractions, and major mineral and fatty acid composition of Burlina cattle breed*

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ABSTRACT

The aim of the present study was to characterise milk of Burlina local cattle breed for traits of technological and nutritional relevance, such as milk coagulation properties (MCP), and protein, major mineral and fatty acid (FA) composition. Burlina is mainly reared in mountain areas of Veneto Region (Italy) and it has been inserted in conservation plans aiming to avoid biodiversity loss and marginal pasture areas abandonment. Eighty-one individual milk samples were collected in four farms. Milk coagulation properties were determined using Formagraph, and protein, mineral and FA composition were analysed in high performance liquid chromatography, inductively coupled plasma optical emission spectrometry and gas chromatography, respectively. Results evidenced good protein percentage (3.38%) and considerable casein content (28.89 mg/mL), as well as a desirable FA profile, with ω-6 to ω-3 ratio of 4.04. Somatic cell score, averaging 3.13, is a trait that should be enhanced through the improvement of farm management. This would have positive effects on MCP. Among milk minerals, the most and less abundant were K (1493.53 mg/kg) and Mg (110.07 mg/kg), respectively. Overall, herd, parity and lactation stage explained moderate to low variation of the studied traits. Results of the present study could be useful to valorise Burlina local breed and preserve biodiversity in marginal areas.

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Local breed; milk coagulation property; casein; mineral; fatty acid

Introduction

The Burlina (BU) is a native cattle breed reared in north-east of Italy, mainly in mountain areas of Veneto Region (Battagin et al. 2010). This local population has been widely appreciated in the past by farmers because of its adaptability to difficult environmental conditions and its good grazing ability. Nevertheless, during the last century the number of animals has decreased drastically. According to official statistics, the population size of the BU breed decreased from 15,000 to few hundreds between 1930 and 1990. This decrease was mainly due to the progressive transition from the extensive to the intensive rearing, and the substitution of the BU breed with the more productive Holstein-Friesian cows (Del Bo et al. 2001). Since the 1980s, the BU has been included in several conservation actions, promoted by public authorities and organisations, aiming to enhance the genetic variability and to encourage the conservation of this native cow in their natural pasture areas (Del Bo et al. 2001; Dalvit et al. 2008; Maretto & Cassandro 2014). These actions allowed to keep the coefficient of inbreeding at low levels: Dalvit et al. (2008) and Battagin et al. (2010) estimated coefficients of inbreeding of less than 1% using microsatellites markers and less than 5% using pedigree information, respectively. Moreover, the conservation programmes stimulated new interest for considering BU breed, particularly in low-input systems where the adoption of specific strategies can make the rearing of this local genetic resource profitable (Pretto et al. 2009). Currently, 428 cows are reared in Italy, particularly in two provinces of north Italy (AIA 2014).

The characterisation of milk in terms of its coagulation ability and quality traits, such as milk protein fractions, major mineral elements and fatty acid (FA) composition could represent a way to add value to BU breed. Milk coagulation properties (MCP) are traits of economic relevance for the dairy sector as they are associated with cheese yield (Cassandro et al. 2016).

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Milk proteins have attracted the attention of scientific community because milk is one of the most suitable sources of protein in the human diet, with positive effects on health (Pereira 2014). Also, it has been demonstrated that milk protein composition influences MCP (Jõudu et al. 2008). Major milk minerals have been widely investigated because of their important effects on human health and for their technological role in cheese production (Toffanin et al. 2015). Finally, milk FA profile has been studied for its relationship with cheese flavour during ripening (Le Quéré 2011), technological properties of butter (MacGibbon & McLennan 1987) and human health. It has been demonstrated that saturated FA have a role in the onset of cardiovascular diseases, obesity and cholesterol level in blood (Siri-Tarino et al. 2010). In addition, these traits are nowadays well predicted by infrared spectroscopy (De Marchi et al. 2014), which implies that the phenotypes can be used for genetic purposes at cow level or to reward/penalise milk in payment systems at dairy industry level.

Therefore, the aim of the present study was to characterise milk of BU cattle breed for MCP, protein composition, major mineral content and FA composition.

Materials and methods

Sample collection, milk chemical composition and coagulation properties

Individual milk samples ($n = 81$) of BU cows from parity 1 to 12 and from 6 to 386 days in milk (DIM) were collected in four herds between March and April 2015. Immediately after sampling, milk was added with preservative (Bronopol, 2-bromo-2-nitropropan-1,3-diol), transferred at 4 °C to the laboratory of the Breeders Association of Veneto Region (ARAV, Padova, Italy) and analysed for fat, protein and lactose content using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark), and somatic cell count (SCC) using a Fossomatic (Foss Electric A/S, Hillerød, Denmark). Values of SCC were transformed to somatic cell score (SCS) to achieve normality and homogeneity of variances through the formula $SCS = 3 + \log_2(SCC/100{,}000)$. Contextually MCP were determined using Formagraph (Foss Electric A/S Hillerød, Denmark), following the method proposed by McMahon and Brown (1982). Measured coagulation properties were rennet coagulation time (RCT, min) and curd firmness 30 min after rennet addition (a_{30}, mm). Samples that did not coagulate within 30 min were considered as non-coagulating milks.

Protein fractions analysis

Milk samples were added with an aqueous solution of guanidine (GND) HCl (6 M GND-HCl, 0.1 M BisTris Buffer, 19.5 mM DTT, 5.37 mM sodium citrate) in a proportion of 1:1 (v:v), incubated at room temperature for 1 h to promote proteins solubilisation and centrifuged for 7 min at room temperature at 16,000 *g*, to promote the separation of fat. An aliquot of soluble fraction was added with a solution containing 4.5 M GND diluted in a solvent consisting of water, acetonitrile, and trifluoroacetic acid (94.9:5.0:0.1; v:v:v), in a proportion of 1:4 (v:v). Samples were finally filtered with a 0.45 nm filter. Analysis of protein fractions was carried out using an high performance liquid chromatography (HPLC) station (Agilent 1260 Series; Agilent Technologies, Santa Clara, CA), equipped with a reversed-phase column C8 (Aeris WIDEPORE XBC8, Phenomenex, 3.6 μm, 300, 250 x 2.1 I.D.). The analysis was conducted following the method proposed by Maurmayr et al. (2013). This method allowed the detection and quantification of α-CN (calculated as sum of α_{s1}-CN and α_{s2}-CN chromatographic peaks), β-CN, κ-CN, β-lactoglobulin A (β-LG A), β-lactoglobulin B (β-LG B), α-lactalbumin (α-LA) and lactoferrin (LF), using internal and external standard and relative calibration curves.

Major minerals analysis

Ca, K, Mg, Na and P contents in milk were determined as described by Toffanin et al. (2015). Briefly, samples were mineralised in nitric acid in a microwave system and inductively coupled plasma optical emission spectrometry, Ciros Vision EOP (SPECTRO Analytical Instruments GmbH, Kleve, Germany) was used to determine Ca at 315.887 nm, K at 766.491 nm, Mg at 280.270 nm, P at 178.287 nm and Na at 589.592 nm. Instrument-operating parameters were optimised for acid solution and calibration standards were matched with nitric acid 5% 'suprapure' grade. The elements to be determined were added from single element solutions (Inorganic Ventures, Christiansburg, VA). The concentration range of the calibration solutions was between 0 and 100 mg/kg for all elements. The accuracy and precision of this method were investigated analysing the certified reference material BCR® - 063R 'Skim milk powder' [(Institute for Reference Materials and Measurements (IRMM), Geel, Belgium)]. The measured and the certified values were in agreement for all the elements (coefficient of determination, $R^2 > 0.99$).

Fatty acids analysis

Milk lipids extraction was performed with accelerated solvent extraction method using Dionex ASE 350 system (Thermo Scientific, Dreieich, Germany) with petroleum ether in isopropanol (2:1) as solvent. Methyl esterification of FA was carried out according to Palmquist and Jenkins (2003) with a basic/acid reaction. Fatty acid separation and quantification were performed by a gas chromatography Agilent 7820A GC System equipped with an automatic sampler G4567A (Agilent Technologies, Santa Clara, CA), a flame ionisation detector and a Supelco Omegawax capillary column (30 m of length, 0.25 mm of inner diameter and a film thickness of 0.25 μm). Temperatures of injector and flame ionisation detector were set at 250 °C. Oven temperature was initially 50 °C for 2 min and then increased at 4 °C/min to 220 °C and held for 18 min. Hydrogen was the carried gas and its flow was set at 1 mL/min with average speed of 21 cm/s. Fatty acids standard Supelco FAME mixC4–C24 #18919-1AMP (Sigma-Aldrich, Castle Hill, Australia) was analysed before gas chromatographic analysis for FA identification. Fatty acids were expressed as g FA/100 g of total identified FA. Groups of FA considered were saturated fatty acids (SFA), calculated as sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0 (and iso and anteiso forms), C14:0, C15:0 (and iso and anteiso forms), C16:0 (and iso form), C17:0 (and iso and anteiso forms), C18:0 (and iso and anteiso forms), C19:0, C20:0, C21:0, C22:0, C23:0, C24:0; monounsaturated fatty acids (MUFA), calculated as sum of C10:1, C12:1, C13:1, C14:1 (and isomers), C15:1, C16:1n9, C16:1n7, C17:1, C18:1 (and isomers), C21:1n9, C22:1n9, C24:1n9; polyunsaturated fatty acids (PUFA) calculated as sum of C18:2 (and isomers), C18:3n6, C18:3n3, C20:2n6, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C22:2n6, C22:5n3, C22:6n3 and CLA; unsaturated fatty acids (UFA) as sum of MUFA and PUFA.

Statistical analysis

Preliminary analysis showed that all traits were normally distributed. For each trait, observations that deviated more than three standard deviations from the mean were considered as outliers and discarded from the dataset. Sources of variation of milk traits were investigated using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effects of herd, parity (4 classes: parity 1, parity 2, parity 3, and parities 4 to 12), and lactation stage (4 classes: 6 to 60 DIM, 61 to 120 DIM, 121 to 180 DIM, and ≥181 DIM). A multiple comparison of means was performed for the fixed effects using Bonferroni's test ($p < .05$).

In the present study, age and season of calving effects were not added to the statistical model to adjust milk characteristics. Season of calving was not included because Burlina herds are almost seasonally calving farms. Cows exploit high summer pastures from May–June to September and thus farmers breed their animals during the first months of the year (January to March) to have cows in mid- to late-lactation during summer. This also means that the majority of the cows (75%) calve in autumn and winter, particularly between October and January. Regarding calving age, we explored the opportunity to group cows of the same parity in 2 or 3 classes of age at calving, in order to account for the effect of, for example, young, medium and old cows within first parity (and the same for other parities). However, the quite small sample size did not allow us to consider this approach because we would have had few observations within each level of age at calving. Also, it is worth noting that that the parity effect already accounts, at least partly, for age at calving effect: on average, the youngest cows were those of first/second parity and the oldest cows were those of later parities.

Results and discussion

Milk chemical composition and coagulation properties

Fat, protein and lactose contents averaged 3.66, 3.38 and 4.74%, respectively (Table 1). Fat content was slightly lower and protein content was greater than values reported by Penasa et al. (2014), who compared three cattle breeds in mixed dairy herds for predicted MCP and composition traits. Fat and lactose percentages were close to those reported for milk of Rendena local cattle breed (Varotto et al. 2015), whereas protein percentage was lower. The SCS averaged 3.13 and this value was greater than that reported by Tiezzi et al. (2015) in a study on the relationships between milk quality and coagulation properties in Italian Holstein–Friesian dairy cattle reared in Veneto region. Means of RCT and a_{30} were 20.01 min and 21.58 mm, respectively (Table 1), which were close to findings of Penasa et al. (2014) on Holstein–Friesian, Brown Swiss and Simmental cows. Milk coagulation properties of BU cows were slightly more favourable than those reported by Visentin et al. (2015) in milk of Alpine Grey breed reared in north-eastern Italian Alps. The coefficient of variation (CV) of a_{30} (55.38%) was more than twice that of RCT (23.77%). About 23% of

Table 1. Descriptive statistics of milk chemical composition, acidity, coagulation traits, protein composition, major minerals and fatty acid (FA) profile.

Trait	No. of samples	Mean	CV, %	Minimum	Maximum
Milk chemical composition					
Fat, %	74	3.66	34.70	1.50	7.30
Protein, %	81	3.38	12.98	2.07	5.10
Lactose, %	79	4.74	5.47	4.12	5.18
SCS	79	3.13	56.25	0.16	7.82
Milk acidity and coagulation traits					
pH	78	6.64	1.10	6.42	6.78
RCT, min	62	20.01	23.77	9.45	29.00
a_{30}, mm	61	21.58	55.38	1.66	50.82
Milk protein composition, mg/mL					
α-CN	80	13.98	20.39	5.94	20.66
β-CN	80	10.20	19.68	6.00	15.37
κ-CN	55	4.71	30.15	0.44	7.61
β-LG A	76	0.82	48.55	0.28	2.90
β-LG B	60	1.44	52.08	0.41	3.64
α-LA	80	0.80	53.60	0.11	1.55
LF	74	0.10	54.41	0.02	0.31
Major milk minerals, mg/kg					
Ca	80	1240.57	12.12	954.55	1657.26
K	81	1493.53	11.36	1034.09	1966.71
Mg	80	110.07	12.46	79.06	148.79
Na	80	500.91	39.75	282.59	988.14
P	80	1013.52	11.44	749.76	1399.67
FA profile, g/100 g of total identified FA					
C14:0	80	11.11	9.70	7.39	13.85
C16:0	81	29.16	10.01	22.27	35.19
C18:0	80	7.95	16.38	5.22	10.93
C18:1n-9	80	16.02	15.55	11.98	25.07
CLA	79	0.66	21.33	0.33	1.04
C23:0	81	6.20	35.70	2.04	10.76
SFA	80	72.02	4.71	62.60	77.52
UFA	78	27.90	12.14	22.48	37.40
MUFA	80	23.53	11.75	19.13	32.69
PUFA	75	4.27	17.78	3.01	6.40
ω-3	81	0.69	26.02	0.32	1.23
ω-6	81	2.79	28.67	1.81	5.93

SCS: somatic cell score, calculated as SCS $=3+\log_2(\text{SCC}/100{,}000)$, where SCC is somatic cell count; RCT: rennet coagulation time; a_{30}: curd firmness 30 min after rennet addition; α-CN: α-casein; β-CN: β-casein; κ-CN: κ-casein; β-LG A: β-lactoglobulin A; β-LG B: β-lactoglobulin B; α-LA: α-lactalbumin; LF: lactoferrin; CLA: conjugated linoleic acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CV: coefficient of variation.

analysed samples did not coagulate within 30 min after rennet addition, and this high percentage could be related with the relatively high milk SCS which has detrimental effects on MCP (Ikonen et al. 2004). None of the fixed effects included in the model was significant in explaining the variation of RCT and a_{30} ($p > .05$). Nevertheless, both traits deteriorated across parities and showed better values in early than late lactation (data not shown), in agreement with findings of Penasa et al. (2014) and Varotto et al. (2015) on milk of Holstein–Friesian cows.

Protein fractions

Descriptive statistics of caseins and whey protein fractions are reported in Table 1. As expected, the most abundant casein fraction was α-CN, which averaged 13.98 mg/mL, followed by β-CN and κ-CN, averaging 10.20 and 4.71 mg/mL, respectively. κ-CN showed the greatest variability, and α-CN and β-CN had almost the same variability. In order of abundance, whey proteins were β-LG B, β-LG A, α-LA and LF, which averaged 1.44, 0.82, 0.80 and 0.10 mg/mL, respectively. These traits exhibited large variability (48.55 to 54.41%). The casein index calculated as ratio between the sum of CN fractions and the sum of all milk protein fractions was close to 90% (Table 1). This finding, considerably high, can be explained through two main reasons. First, the HPLC method did not allow the detection and the quantification of some whey protein fractions, such as immunoglobulins and bovine serum albumin, as well as non-protein nitrogen, determining an overestimation of casein index. Second, Pacini et al. (2008) highlighted a greater frequency of κ-CN B allele in BU cattle breed, which is associated with greater casein content (Vallas et al. 2012). De Marchi et al. (2009), Bonfatti et al. (2011) and Niero et al. (2016) reported values of casein index that were similar to the one calculated in the present study.

Significance of fixed effects included in the model for caseins and whey protein fractions is reported in Table 2. Coefficients of determination ranged from 0.13 (β-LG A) to 0.54 (β-LG B and α-LA), suggesting that a significant part of the total phenotypic variance of traits is determined by effects other than those considered in the present study. Herd effect was significant ($p < .05$) for all protein fractions with the exception of α-CN and LF. Parity effect was significant ($p < .05$) for α-CN, α-LA and LF, and lactation stage was significant ($p < .05$) only for β-CN and κ-CN.

Tables 3 and 4 report the least squares means of caseins and whey proteins across parity and lactation stage, respectively. Concentration of casein fractions across parity was quite stable: only α-CN content was significantly lower for third than first and second parity cows ($p < .05$; Table 3). Caseins showed higher variability across different lactation stages: both κ-CN and β-CN concentrations were significantly greater in late than early lactation ($p < .05$; Table 4). Despite not significant, this result was confirmed also for α-CN, in agreement with findings of Ng-Kwai-Hang et al. (1982). Regarding whey proteins, LF was more abundant in third than other parity cows ($p < .05$; Table 3). Finally no significant differences were detected across lactation for whey proteins (Table 4).

Major milk minerals

Descriptive statistics of major milk minerals are reported in Table 1. The most abundant milk mineral

Table 2. F-value and significance of fixed effects included in the analysis for milk protein composition, major minerals and fatty acid (FA) profile.

Trait	Herd	Parity	Lactation stage	R^2	RMSE
Milk protein composition, mg/mL					
α-CN	0.75	3.80[a]	2.10	0.23	2.65
β-CN	3.56[a]	0.82	6.89[c]	0.32	1.76
κ-CN	8.84[c]	1.13	4.12[a]	0.21	1.17
β-LG A	2.73[a]	0.22	0.21	0.13	0.35
β-LG B	16.19[c]	0.59	1.43	0.54	0.55
α-LA	22.82[c]	3.15[a]	1.23	0.54	0.15
LF	1.91	5.83[b]	0.45	0.26	0.05
Major milk minerals, mg/kg					
Ca	0.96	0.93	1.10	0.16	144.71
K	1.41	1.63	1.91	0.21	156.31
Mg	1.32	0.22	2.87	0.20	13.03
Na	11.29[c]	3.16[b]	1.58	0.47	155.31
P	0.95	2.29	2.17	0.22	107.01
FA profile, g/100 g of total identified FA					
C14:0	0.77	3.08[a]	5.54[b]	0.24	1.00
C16:0	12.22[c]	5.79[b]	3.66[a]	0.39	2.43
C18:0	0.72	2.95[a]	4.75[b]	0.26	1.18
C18:1n-9	11.29[c]	5.34[b]	3.94[a]	0.38	2.10
CLA	8.38[c]	1.76	1.50	0.31	0.13
C23:0	1.70	0.47	0.72	0.14	2.17
SFA	18.23[c]	5.09[b]	2.00	0.47	2.65
UFA	19.23[c]	4.46[b]	2.09	0.48	2.62
MUFA	11.95[c]	4.68[b]	2.56	0.37	2.34
PUFA	27.90[c]	1.33	0.87	0.60	0.52
ω-3	45.41[c]	1.25	1.53	0.67	0.11
ω-6	27.3[c]	2.26	0.43	0.58	0.55

α-CN: α-casein; β-CN: β-casein; κ-CN: κ-casein; β-LG A: β-lactoglobulin A; β-LG B: β-lactoglobulin B; α-LA: α-lactalbumin; LF: lactoferrin; CLA: conjugated linoleic acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; RMSE: root mean square error.
[a]$p < .05$.
[b]$p < .01$.
[c]$p < .001$.

was K (1493.53 mg/kg), followed by Ca (1240.57 mg/kg), P (1013.52 mg/kg), Na (500.91 mg/kg) and Mg (110.07 mg/kg). These values are consistent with those summarised by Gaucheron (2005) in a review on cow milk minerals. Also, Ca content was close to findings of Chiofalo et al. (2000) in a study on Modicana local cattle breed. The most variable milk mineral was Na (CV =39.75%), whereas the most stable were P (CV =11.44%) and K (CV =11.36%).

Herd and parity effects were significant ($p < .05$) only for Na content, whereas lactation stage did not influence the mineral composition of milk (Table 2). The R^2 of Na was moderate (0.47), and that of Ca, K, Mg and P contents was low (<0.25). Despite not significant, results indicate that Ca concentration peaked in second lactation cows and decreased in third and later parities (Table 3). The deteriorating trend of Ca concentration in multiparous cows was probably due to a minor physiological efficiency of parathyroid hormone, which is involved in the mobilisation of Ca from bones to blood and subsequently to milk (Kume & Tanabe 1993). Phosphorus, which is known to be strongly and positively correlated with Ca, showed a trend across parity that was very similar to Ca (Toffanin et al. 2015). Sodium had the greatest concentration in third and fourth and later parity cows, whereas K content was greater in first and second lactation animals (Table 3). The high levels of Na and low levels of K could be associated with high SCS in older than younger cows (data not shown), as suggested by Summer et al. (2009). The trend for Ca, P, Na and Mg across lactation (Table 4) is somewhat opposite to the typical milk lactation curve, hypothesising a dilution effect for these elements in milk.

Fatty acid composition

Major FA identified in milk samples were C14:0, C16:0, C18:0 and C18:1n9, averaging 11.11, 29.16, 7.95 and 16.02 g/100 g of total identified FA, respectively (Table 1). Mean values for C18:0 and C18:1n9 were slightly lower than values reported by Auldist et al. (2004) in Friesian and Jersey breeds. Average SFA, UFA, MUFA and PUFA were 72.02, 27.90, 23.53 and 4.27 g/100 g of total identified FA, respectively, and means for ω-3 and ω-6 were 0.69 and 2.79 g/100 of total identified FA, respectively (Table 1). In the present study, UFA content resulted slightly lower than that reported by Chiofalo et al. (2000) on milk of Modicana native cattle breed, and ω-3 content was similar and ω-6 was greater than findings of Lindmark-Månsson (2008) in a study on FA composition of Swedish dairy milk. The ratio between ω-6 and ω-3 of the present work was 4.04. As reported by Yashodhara et al. (2009), different ω-6 to ω-3 ratios revealed different positive effects on human health; in particular, a diet with ω-6 to ω-3 ratio close to 4 is recommended for prevention of cardiovascular diseases.

Herd effect was statistically significant in explaining the variation of all FA groups and almost all individual FA, with the exception of C14:0, C18:0 and C23:0 (Table 2). Parity effect was significant for C14:0, C16:0, C18:0, C18:1n9, SFA, UFA and MUFA, and the effect of lactation stage was significant only for C14:0, C16:0, C18:0 and C18:1n9. The R^2 of FA ranged from 0.14 (C23:0) to 0.67 (ω-3), suggesting that effects included in the statistical model had different impact in explaining the variation of milk FA composition.

The C16:0 content was greater ($p < .05$) in second and fourth and later parities than in primiparous cows (Table 3). This finding is consistent with Miller et al. (2006), who demonstrated that the mammary gland of primiparous cows is metabolically less active than that of later parity animals, with lower activity of FA synthase, and thus with lower C16:0 content. On the

Table 3. Least squares means (standard errors) of milk protein composition, major milk minerals and fatty acid (FA) profile across parity.

	Parity			
Trait	First	Second	Third	Fourth and later
Milk protein composition, mg/mL				
α-CN	14.63 (0.68)[a]	15.88 (0.70)[a]	12.71 (0.75)[b]	14.55 (0.70)[a,b]
β-CN	10.85 (0.45)	10.59 (0.47)	10.26 (0.50)	9.90 (0.46)
κ-CN	4.55 (0.37)	3.99 (0.39)	3.53 (0.49)	3.99 (0.46)
β-LG A	0.77 (0.09)	0.84 (0.10)	0.87 (0.10)	0.73 (0.10)
β-LG B	1.65 (0.21)	1.65 (0.18)	1.51 (0.19)	1.36 (0.15)
α-LA	0.70 (0.04)	0.69 (0.04)	0.83 (0.04)	0.82 (0.04)
LF	0.09 (0.01)[a]	0.08 (0.01)[a]	0.15 (0.01)[b]	0.09 (0.01)[a]
Major milk minerals, mg/kg				
Ca	1209.90 (39.53)	1288.43 (38.95)	1244.50 (41.85)	1225.93 (38.63)
K	1470.10 (39.84)	1534.99 (41.46)	1411.08 (44.27)	1460.20 (40.68)
Mg	108.61 (3.56)	109.05 (3.51)	110.98 (3.77)	107.58 (3.48)
Na	426.05 (39.60)	434.79 (41.19)	544.87 (44.87)	566.92 (40.43)
P	1047.74 (27.52)	1064.62 (28.41)	990.62 (30.33)	977.85 (27.85)
FA profile, g/100 g of total identified FA				
C14:0	10.33 (0.26)	11.23 (0.26)	10.64 (0.28)	11.18 (0.26)
C16:0	26.94 (0.62)[a]	30.03 (0.65)[b]	28.64 (0.69)[a,b]	29.96 (0.63)[b]
C18:0	8.34 (0.30)	7.66 (0.31)	8.44 (0.34)	7.46 (0.31)
C18:ln-9	17.74 (0.56)[a]	15.25 (0.56)[b]	16.23 (0.59)[a,b]	15.05 (0.55)[b]
CLA	0.72 (0.03)	0.65 (0.03)	0.63 (0.04)	0.64 (0.03)
C23:0	6.66 (0.55)	6.29 (0.58)	7.18 (0.61)	6.96 (0.56)
SFA	69.72 (0.70)[a]	72.78 (0.70)[b]	72.42 (0.75)[b]	73.13 (0.69)[b]
UFA	29.96 (0.71)[a]	27.17 (0.70)[b]	27.44 (0.76)[a,b]	26.74 (0.70)[b]
MUFA	25.36 (0.62)[a]	22.72 (0.62)[b]	23.39 (0.66)[a,b]	22.50 (0.61)[b]
PUFA	4.60 (0.14)	4.39 (0.14)	4.22 (0.15)	4.31 (0.14)
ω-3	0.82 (0.03)	0.75 (0.03)	0.76 (0.03)	0.75 (0.03)
ω-6	3.01 (0.14)	2.82 (0.15)	2.51 (0.16)	2.65 (0.14)

α-CN: α-casein; β-CN: β-casein; κ-CN: κ-casein; β-LG A: β-lactoglobulin A; β-LG B: β-lactoglobulin B; α-LA: α-lactalbumin; LF: lactoferrin; CLA: conjugated linoleic acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[a,b]Least squares means with different superscripts within a row are significantly different according to Bonferroni's correction ($p < .05$).

Table 4. Least squares means (standard errors) of milk protein composition, major milk minerals and fatty acid (FA) profile across lactation stage.

	Lactation stage, d			
Trait	5 to 60	61 to 120	121 to 180	>180
Milk protein composition, mg/mL				
α-CN	14.09 (0.94)	13.36 (0.57)	14.57 (0.61)	15.75 (0.91)
β-CN	9.60 (0.63)[a]	9.27 (0.38)[a]	10.56 (0.40)[a,b]	12.17 (0.60)[b]
κ-CN	3.22 (0.75)[a]	3.55 (0.31)[a]	3.96 (0.37)[a]	5.33 (0.44)[b]
β-LG A	0.78 (0.13)	0.86 (0.08)	0.80 (0.08)	0.79 (0.13)
β-LG B	1.44 (0.23)	1.44 (0.15)	1.35 (0.13)	1.93 (0.25)
α-LA	0.78 (0.05)	0.79 (0.03)	0.80 (0.04)	0.83 (0.05)
LF	0.11 (0.02)	0.10 (0.01)	0.09 (0.01)	0.12 (0.02)
Major milk minerals, mg/kg				
Ca	1226.54 (52.17)	1201.66 (31.22)	1228.56 (33.74)	1312.01 (53.72)
K	1437.35 (55.72)	1513.80 (33.56)	1529.94 (35.86)	1395.25 (53.42)
Mg	104.21 (4.70)	103.98 (2.81)	110.20 (3.04)	117.84 (4.84)
Na	447.41 (55.42)	474.72 (33.35)	457.88 (35.85)	592.63 (53.11)
P	987.97 (38.16)	987.20 (23.00)	1012.67 (24.83)	1093.00 (36.62)
FA profile, g/100 g of total identified FA				
C14:0	9.88 (0.38)[a]	11.04 (0.21)[b]	11.52 (0.23)[b]	10.95 (0.34)[a,b]
C16:0	27.64 (0.87)[a]	29.62 (0.52)[a]	30.35 (0.56)[b]	27.97 (0.83)[a]
C18:0	9.16 (0.42)[a]	7.89 (0.26)[b]	7.64 (0.27)[b]	7.22 (0.40)[b]
C18:ln-9	17.74 (0.80)[a]	15.64 (0.45)[a,b]	14.84 (0.48)[b]	16.04 (0.72)[a]
CLA	0.60 (0.05)	0.65 (0.03)	0.66 (0.03)	0.73 (0.04)
C23:0	7.09 (0.77)	6.33 (0.47)	6.31 (0.50)	7.37 (0.74)
SFA	70.69 (1.01)	72.56 (0.57)	73.18 (0.61)	71.61 (0.91)
UFA	29.08 (1.01)	27.12 (0.59)	26.68 (0.62)	28.43 (0.90)
MUFA	24.77 (0.89)	23.11 (0.50)	22.29 (0.54)	23.80 (0.80)
PUFA	4.40 (0.19)	4.22 (0.12)	4.38 (0.13)	4.51 (0.18)
ω-3	0.78 (0.04)	0.73 (0.02)	0.76 (0.03)	0.81 (0.04)
ω-6	2.78 (0.20)	2.63 (0.12)	2.77 (0.13)	2.80 (0.19)

α-CN: α-casein; β-CN: β-casein; κ-CN: κ-casein; β-LG A: β-lactoglobulin A; β-LG B: β-lactoglobulin B; α-LA: α-lactalbumin; LF: lactoferrin; CLA: conjugated linoleic acids; SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[a,b]Least squares means with different superscripts within a row are significantly different according to Bonferroni's correction ($p < .05$).

other hand, C18:1n9 showed the greatest values for first parity cows, and the lowest for second and fourth and later parity animals ($p < .05$). Saturated FA content was significantly lower in primiparous than older cows, whereas UFA and MUFA exhibited an opposite trend ($p < .05$; Table 3). The effect of parity on milk FA composition has been investigated in literature, but results are somewhat controversial. Secchiari et al. (2003) and Kgwatalala et al. (2009) did not observe significant effects of parity on milk FA composition. On the opposite, Kelsey et al. (2003) estimated a significant parity effect on milk FA composition of US and Canadian Holsteins, respectively.

Regarding FA variation across lactation, C14:0 and C16:0 showed similar trends, with the lowest concentration in early and the greatest in mid-lactation, whereas C18:0 was greater at the beginning than late lactation ($p < .05$; Table 4). Finally, C18:1n9 exhibited the greatest value in early lactation and the lowest in mid lactation. Results for C16:0 and C18:1n9 were in agreement with findings of Samková et al. (2012). Conjugated linoleic acids content did not change across lactation, supporting previous results of Kelsey et al. (2003). Albeit not significant, ω-3 and ω-6 showed the greatest concentrations in early and late lactation.

Conclusions

Results of the present study allowed the characterisation of milk coagulation ability, protein fractions, major mineral composition and FA profile of BU cow. Milk coagulation traits were not optimal, probably because of the relatively high SCS, and thus more efforts are needed to improve rearing and management conditions. The casein index was desirable and this result is interesting for cheese production. A favourable ω-6 to ω-3 ratio was found, which may have positive effects on human health. Parity and lactation stage of cows had a significant effect mainly on milk FA profile, and partially on protein composition. Findings of the present study allowed a better understanding of the peculiarities of BU and they could be useful to valorise this local breed and to maintain biodiversity in marginal areas.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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