

# Study of milk protein composition and coagulation properties of Burlina local cattle breed

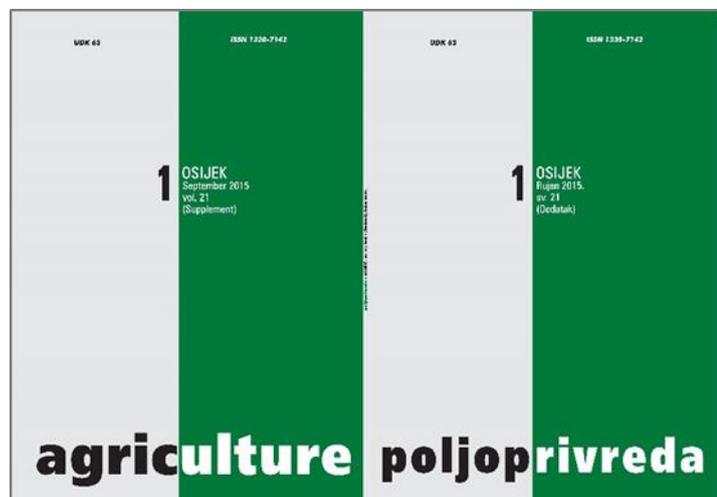
**Niero, G., Penasa, M., De Marchi, M., Visentin, G., Cassandro, M.**

**Poljoprivreda/Agriculture**

ISSN: 1848-8080 (Online)

ISSN: 1330-7142 (Print)

<http://dx.doi.org/10.18047/poljo.21.1.sup.23>



**Poljoprivredni fakultet u Osijeku, Poljoprivredni institut Osijek**

Faculty of Agriculture in Osijek, Agricultural Institute Osijek

# STUDY OF MILK PROTEIN COMPOSITION AND COAGULATION PROPERTIES OF BURLINA LOCAL CATTLE BREED

Niero, G., Penasa, M., De Marchi, M., Visentin, G., Cassandro, M.

Original scientific paper

## SUMMARY

***Burlina is a dual-purpose cattle breed, mainly reared in mountain areas of Veneto Region (Italy). The aim of this study was to investigate milk protein composition and milk coagulation properties (MCP) of Burlina breed. A total of 80 individual milk samples were collected and milk casein fractions were detected and quantified with reversed-phase high-performance liquid chromatographic analysis whereas MCP were determined with Formagraph. Sources of casein fractions variation and MCP were investigated using a linear model including herd, parity, and days in milk as fixed effects. Casein fractions showed increasing concentrations across days in milk, but not specific trends across parities. Milk coagulation properties exhibited better values in early than late lactation stages. Also, they deteriorated across parities.***

***Key-words: local breed, casein fraction, milk coagulation property***

## INTRODUCTION

Burlina is a native dual-purpose 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, but during the last century the number of animals has decreased drastically. Official statistics report that the population size decreased from 15,000 animals in 1930 to few hundreds in 1990, mainly because of the progressive substitution with the more productive Holstein-Friesian cows (Del Bo et al., 2001). Since the 1980s, the Burlina has been inserted in a conservation program, promoted by public authorities and organisations. Aim of this program is to enhance the genetic variability and to encourage the conservation of a native animal genetic resource to preserve pastures in marginal and fragile environments (Del Bo et al., 2001; Dalvit et al., 2008).

The present study aims to characterize milk from Burlina cattle breed, giving particular emphasis to milk casein fractions and milk coagulation properties (MCP). There is scientific evidence that casein fractions and MCP are useful information for cheese processing, yield and quality, especially in countries where dairy industry is based on traditional cheeses (Cassandro, 2003).

## MATERIAL AND METHODS

### Data

Individual milk samples (n=80) of Burlina cows from parity 1 to 12 and from 6 to 386 days in milk (DIM) were collected in 4 herds between March and April 2015. Immediately after sampling, milks were added with preservative, transferred at 4°C to the laboratory of the Breeders Association of Veneto Region (Padova, Italy) and analyzed for milk chemical composition using a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark) as well as somatic cell count (SCC) using a Fossomatic (Foss Electric A/S, Hillerød, Denmark). Following the recommendations of the International Committee for Animal Recording (ICAR, 2014), values of fat and protein contents, outside a range of 1.5 to 9% and 1 to 7%, respectively, were identified as outliers. Values of SCC were transformed to somatic cell score (SCS) through the formula  $SCS = 3 + \log_2(SCC/100,000)$ . Milk coagulation properties (MCP), namely rennet coagulation time (RCT) and curd firmness ( $a_{30}$ ), were determined by Formagraph (Foss Electric A/S, Hillerød, Denmark). An aliquot of each sample was transferred to

(1) M.Sc. Giovanni Niero (giovanni.niero@studenti.unipd.it), Assistant Prof. Mauro Penasa, Assoc. Prof. Massimo De Marchi, M.Sc. Giulio Visentin, Prof. Dr. Martino Cassandro - University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment, Viale dell'Università 16, 35020 Legnaro (PD), Italy

the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy). Analysis of casein fractions was carried out using a high-performance liquid chromatography station, Agilent 1260 Series (Agilent Technologies, Santa Clara, CA, USA), equipped with a reversed-phase analytical column C8 (Aeris WIDEPOR XBC8, Phenomenex, 3,6  $\mu$ m, 300., 250 x 2,1 I.D.). The analysis was conducted following the method proposed by Maurmayr et al. (2013).

### Statistical analysis

The normal distribution of studied traits was checked using Shapiro-Wilk's test. Pearson correlations between traits were estimated through the CORR procedure of SAS (SAS Institute Inc., Cary, NC, USA). Sources of variation of casein fractions and MCP were investigated using the GLM procedure of SAS. The model included the fixed effects of herd, parity (4 classes: parity 1, parity 2, parity 3, and parities 4 to 8), and DIM (4 classes: 6 to 60 d, 61 to 120 d, 121 to 180 d, and  $\geq$ 181 d). A multiple comparison of means was performed for the fixed effects using Bonferroni's test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Descriptive statistics and significance of fixed effects

Table 1 shows descriptive statistics of quality traits, casein fractions and technological characteristics of individual milk samples. The most abundant casein fraction was  $\alpha$ -casein ( **$\alpha$ -Cn**), which averaged  $13.98 \pm 2.85$  mg/mL, followed by  $\beta$ -casein ( **$\beta$ -Cn**) and  $\kappa$ -casein ( **$\kappa$ -Cn**), averaging  $10.21 \pm 2.01$  and  $4.71 \pm 1.42$  mg/mL, respectively. Contents of casein fractions found in the present work were similar to those reported by De Marchi et al. (2009) in a study aiming to characterise milk composition of the Simmental breed. Concerning MCP, RCT and  $a_{30}$  was averaged  $20.08 \pm 4.77$  min and  $21.58 \pm 11.95$  mm, respectively.

Significance of fixed effects included in the analysis of casein fractions and milk technological traits is reported in Table 2. The coefficient of determination ranged from 0.15 (RCT) to 0.37 ( $\kappa$ -Cn), suggesting that there is an important portion of the phenotypic variance not explained by the factors included in the statistical model for the studied traits. The  $\kappa$ -Cn and  $\beta$ -Cn fractions were significantly ( $P < 0.05$ ) affected by herd and DIM whereas  $\alpha$ -Cn was influenced only by parity ( $P < 0.05$ ). Herd was an important factor in explaining the variation of pH and SCS, along with DIM for pH and parity for SCS ( $P < 0.05$ ).

**Table 1. Descriptive statistics<sup>(1)</sup> of milk quality, casein fractions and milk technological traits**

Trait <sup>(2)</sup>	N	Mean	SD	Minimum	Maximum
Fat, %	74	3.66	1.27	1.50	7.30
Protein, %	80	3.38	0.44	2.07	5.10
Casein, %	80	2.63	0.36	1.60	4.04
$\kappa$ -Cn, mg/mL	55	4.71	1.42	0.44	7.61
$\alpha$ -Cn, mg/mL	80	13.98	2.85	5.94	20.66
$\beta$ -Cn, mg/mL	80	10.21	2.01	6.00	15.37
RCT, min	61	20.08	4.77	9.45	29.00
$a_{30}$ , mm	61	21.58	11.95	1.66	50.82
pH	80	6.63	0.10	6.16	6.88
SCS	78	3.16	1.75	0.16	7.82

<sup>(1)</sup> SD = standard deviation; <sup>(2)</sup>  $\kappa$ -Cn =  $\kappa$ -casein;  $\alpha$ -Cn =  $\alpha$ -casein;  $\beta$ -Cn =  $\beta$ -casein; RCT = rennet coagulation time;  $a_{30}$  = curd firmness; SCS = somatic cell score

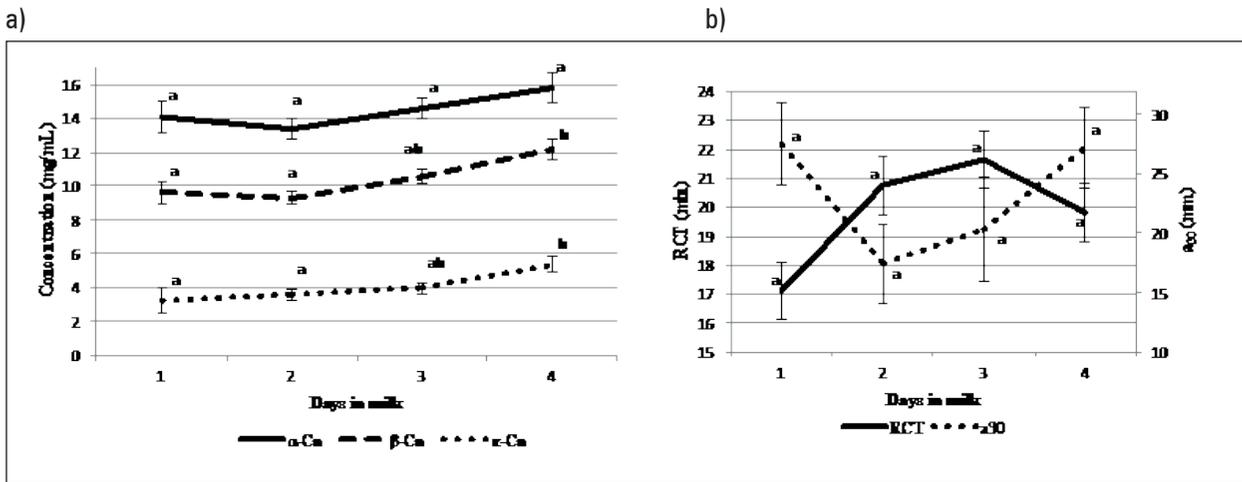
**Table 2. F-value and significance of fixed effects included in the analysis of casein fractions and milk technological traits**

Trait <sup>(1)</sup>	Herd	Parity	DIM <sup>(2)</sup>	R <sup>2</sup>	RMSE <sup>(3)</sup>
$\kappa$ -Cn, mg/mL	8.84***	1.13	4.12*	0.37	1.22
$\alpha$ -Cn, mg/mL	0.75	3.79*	2.10	0.23	2.65
$\beta$ -Cn, mg/mL	3.56*	0.82	6.89***	0.32	1.76
RCT, min	0.68	1.10	1.82	0.15	4.77
$a_{30}$ , mm	0.52	1.53	1.95	0.17	11.81
pH	6.66***	2.25	3.08*	0.27	0.09
SCS	4.20**	5.66**	1.73	0.35	1.50

<sup>(1)</sup>  $\kappa$ -Cn =  $\kappa$ -casein;  $\alpha$ -Cn =  $\alpha$ -casein;  $\beta$ -Cn =  $\beta$ -casein; RCT = rennet coagulation time;  $a_{30}$  = curd firmness; SCS = somatic cell score; <sup>(2)</sup> DIM = days in milk; <sup>(3)</sup> RMSE = root mean square error; Statistical significance is given as: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

### Least squares means

Figure 1 depicts the least squares means of milk casein fractions and MCP across DIM. In particular,  $\kappa$ -Cn and  $\beta$ -Cn concentrations were more abundant in late than early lactation stages ( $P < 0.05$ ). Despite being not significant, this result was confirmed also for  $\alpha$ -Cn. Similar lactation variation of casein fraction contents was observed by Ng-Kwai-Hang et al. (1982). Rennet coagulation time resulted shorter in early lactation, and  $a_{30}$  exhibited the best values at the beginning and end of the lactation. Despite the trends for MCP across DIM that were not statistically significant ( $P > 0.05$ ), they were very similar to findings of Penasa et al. (2014) and Varotto et al. (2015) on milk of Holstein-Friesian cows.

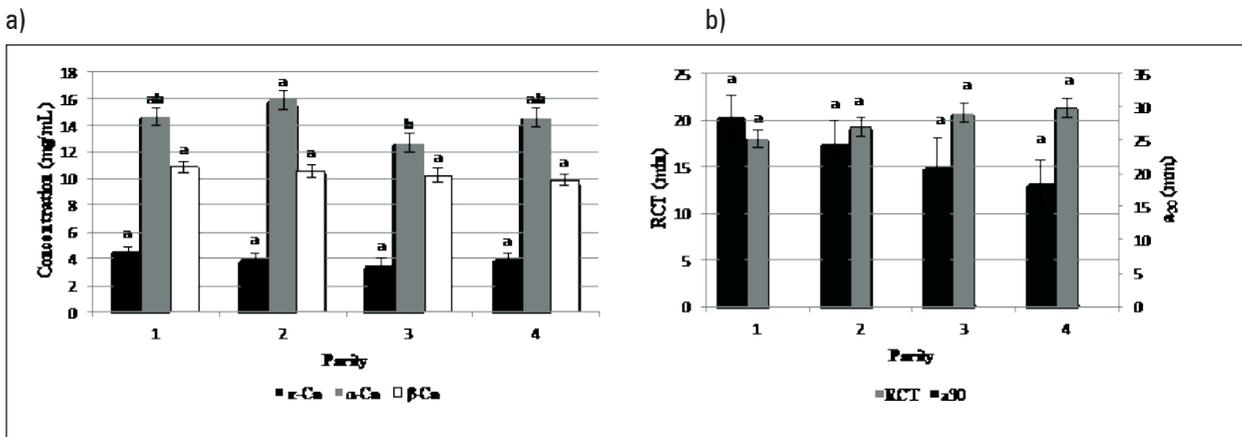


<sup>(1)</sup> κ-Cn = κ-casein; α-Cn = α-casein; β-Cn = β-casein; <sup>(2)</sup> RCT = rennet coagulation time; a<sub>30</sub> = curd firmness

**Figure 1. Least squares means of (a) milk casein fractions<sup>(1)</sup> and (b) milk coagulation properties<sup>(2)</sup> across days in milk. Least squares means with different letters across days in milk for a given trait mean that they are significantly different according to Bonferroni's correction (P<0.05)**

Figure 2 depicts the least squares means of milk casein fractions and MCP across parities. Concentration of casein fractions was quite stable and did not show particular trends across parities. Only α-Cn exhibited different concentration (P<0.05) in milk from the second and third parity cows. Albeit not significant (P>0.05), RCT and a<sub>30</sub> deteriorated from the first to fourth and later

parities, suggesting that MCP were more favourable in primiparous than multiparous cows. This trend was observed also in other studies (Tyrisevä et al., 2003; Penasa et al., 2014; Varotto et al., 2015). However, Ikonen et al. (2004) reported lower values of a<sub>30</sub> in milk of primiparous than multiparous cows.



<sup>(1)</sup> κ-Cn = κ-casein; α-Cn = α-casein; β-Cn = β-casein; <sup>(2)</sup> RCT = rennet coagulation time; a<sub>30</sub> = curd firmness

**Figure 2. Least squares means of (a) milk casein fractions<sup>(1)</sup> and (b) milk coagulation properties<sup>(2)</sup> across parities. Least squares means with different letters across parities for a given trait mean that they are significantly different according to Bonferroni's correction (P<0.05)**

**CONCLUSION**

The present study is the first contribution to the quantification of casein fractions in Burlina cattle population. Casein fractions showed the highest concentration in late lactation, and MCP showed better values in early lactation. Moreover, MCP deteriorated across parities. Overall, the comparison of results from the present study with the scientific literature suggests that Burlina

has similar casein composition and MCP of other cattle breeds, such as Simmental and Holstein-Friesian, thus indicating that small-sized local breeds could be interesting for traits of economic importance.

**ACKNOWLEDGEMENT**

The authors would like to thank Sandro Longo (Breeders Association of Veneto Region, Padova, Italy)

and Ph.D. Roberto Santomaso (Breeders Association of Treviso Province) for technical support. The research was funded by the Project Burbacco (PSR 2007 – 2013, Mis. 124 – Aiuto N° 2308615).

## REFERENCES

- Battagin, M., Penasa, M., Pretto, D., Cassandro, M. (2010): Pedigree analysis of Burlina cattle population. *Acta Agraria Kaposváriensis*, 14(2):161-165.
- Cassandro, M. (2003): Status of milk production and market in Italy. *Agriculturae Conspectus Scientificus*, 68: 65-69.
- Dalvit, C., De Marchi, M., Dal Zotto, R., Zanetti, E., Meuwissen, T., Cassandro, M. (2008): Genetic characterization of the Burlina cattle breed using microsatellites markers. *Journal of Animal Breeding and Genetics*, 125: 137-144.  
doi: <http://dx.doi.org/10.1111/j.1439-0388.2007.00707.x>
- Del Bo, L., Polli, M., Longeri, M., Ceriotti, G., Looft, C., Barre-Dirie, A., Dolf, G., Zanotti, M. (2001): Genetic diversity among some cattle breeds in the Alpine area. *Journal of Animal Breeding and Genetics*, 118: 317-325.  
doi: <http://dx.doi.org/10.1046/j.1439-0388.2001.00306.x>
- De Marchi, M., Bonfatti, V., Cecchinato, A., Di Martino, G., Carnier, P. (2009): Prediction of protein composition of individual cow milk using mid-infrared spectroscopy. *Italian Journal of Animal Science*, 8 (Suppl. 2): 399-401.
- ICAR (International Committee for Animal Recording). (2012): ICAR recording guidelines. Accessed July 21, 2015. [http://www.icar.org/pages/recording\\_guidelines.htm](http://www.icar.org/pages/recording_guidelines.htm).
- Ng-Kwai-Hang, K.F., Hayes, J.F., Moxley, J.E., Monardes, H.G. (1982): Environmental influences on protein content and composition of bovine milk. *Journal of Dairy Science*, 65:1993-1998.  
doi: [http://dx.doi.org/10.3168/jds.S0022-0302\(82\)82449-1](http://dx.doi.org/10.3168/jds.S0022-0302(82)82449-1)
- Ikonen, T., Morri, S., Tyrisevä, A.M., Ruottinen, O., Ojala, M. (2004): Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. *Journal of Dairy Science*, 87: 458-467.  
doi: [http://dx.doi.org/10.3168/jds.S0022-0302\(04\)73185-9](http://dx.doi.org/10.3168/jds.S0022-0302(04)73185-9)
- Maurmayr, A., Cecchinato, A., Grigoletto, L., Bittante, G. (2013): Detection and quantification of  $\alpha_{S1}$ -,  $\alpha_{S2}$ -,  $\beta$ -,  $\kappa$ -casein,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and lactoferrin in bovine milk by reverse-phase high-performance liquid chromatography. *Agriculturae Conspectus Scientificus*, 78: 201-205.
- Penasa, M., Tiezzi, F., Sturaro, A., Cassandro, M., De Marchi, M. (2014): A comparison of the predicted coagulation characteristics and composition of milk from multi-breed herds of Holstein-Friesian, Brown Swiss and Simmental cows. *International Dairy Journal*, 35:6-10.  
doi: <http://dx.doi.org/10.1016/j.idairyj.2013.10.004>
- Tyrisevä, A.M., Ikonen, T., Ojala, M. (2003): Repeatability estimates for milk coagulation traits and non-coagulation of milk in Finnish Ayrshire cows. *Journal of Dairy Research*, 70: 91-98.  
doi: <http://dx.doi.org/10.1017/S0022029902005939>
- Varotto, A., De Marchi, M., Penasa, M., Cassandro, M. (2015): A comparison of milk clotting characteristics and quality traits of Rendena and Holstein-Friesian cows. *Italian Journal of Animal Science*, 14:202-206.  
doi: <http://dx.doi.org/10.4081/ijas.2015.3768>

(Received on 29 April 2015; accepted on 21 July 2015)