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Short communication: Factors affecting coagulation properties of Mediterranean buffalo milk

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ABSTRACT

The aim of this study was to investigate sources of variation of milk coagulation properties (MCP) of buffalo cows. Individual milk samples were collected from 200 animals in 5 herds located in northern Italy from January to March 2010. Rennet coagulation time (RCT, min) and curd firmness after 30 min from rennet addition (a_{30}, mm) were measured using the Formagraph instrument (Foss Electric, Hillerød, Denmark). In addition to MCP, information on milk yield, fat, protein, and casein contents, pH, and somatic cell count (SCC) was available. Sources of variation of RCT and a_{30} were investigated using a linear model that included fixed effects of herd, days in milk (DIM), parity, fat content, casein content (only for a_{30}), and pH. The coefficient of determination was 51% for RCT and 48% for a_{30}. The most important sources of variation of MCP were the herd and pH effects, followed by DIM and fat content for RCT, and casein content for a_{30}. The relevance of acidity in explaining the variation of both RCT and a_{30}, and of casein content in explaining that of a_{30}, confirmed previous studies on dairy cows. Although future research is needed to investigate the effect of these sources of variation on cheese yield, findings from the present study suggest that casein content and acidity may be used as indicator traits to improve technological properties of buffalo milk.
Key words: buffalo, milk coagulation property, production, quality trait

Short Communication

Buffalo (*Bubalus bubalis*) farming is gaining interest in Italy because demand for the traditional Mozzarella cheese has increased and the product is well paid by the market (Addeo et al., 2007). Mozzarella production is traditionally located in the south of the country; however, during the last years the number of animals has grown in the north because of the high price paid for milk and the potential role that buffalo may play in differentiating products and increasing competitiveness in the market.

The assessment of milk coagulation properties (**MCP**) can be performed through different instruments such as the Formagraph (Foss Electric, Hillerød, Denmark) or computerized renneting meter, which provides measures of rennet coagulation time (**RCT**, min) and curd firmness after 30 min from rennet addition (**a_{30}**, mm), but also using alternative systems, based on optical, thermal, and vibrational methods (O'Callaghan et al., 2002; Kübarsepp et al., 2005; Cecchinato et al., 2009; De Marchi et al., 2009). Only a few researchers have dealt with MCP of buffalo milk and the factors affecting its variation (e.g., Bartocci et al., 2002; Ariota et al., 2007; Potena et al., 2007b). Therefore, the aim of this study was to investigate sources of variation of MCP using individual milk samples of buffalo cows.

Two hundred Mediterranean buffalo cows were sampled once in 5 herds located in northern Italy (4 in Veneto and 1 in Friuli-Venezia Giulia region) from January to March 2010. Individual milks were collected, without preservative, during the morning milking, stored in portable refrigerators (4°C), transferred to the milk quality laboratory of the Department of Animal Science (University of Padova, Legnaro, Italy), and analyzed for RCT and a_{30} within 3 h from sampling. Measures of MCP were obtained using the Formagraph instrument (Foss Electric). The working principle of the device and the typical diagram produced are fully described in McMahon and Brown (1982).

Milk samples (10 mL) were heated to 35°C, and 200 μL of rennet (Hansen standard 160 with 63% chymosin and 37% pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted to 1.6% in distilled water was added to the milk. The analysis ended within 30 min from the addition of the clotting enzyme and provided measurements of RCT (min), defined as the time interval between the addition of the enzyme and the beginning of the coagulation process, and a_{30} (mm), defined as the width of the diagram 30 min after the addition of rennet. Only one sample did not coagulate within 30

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Table 1. Descriptive statistics of milk coagulation properties, and production and quality traits of buffalo cows (n = 200)[1]

Trait[2]	Mean	P5	P95	CV, %
Milk coagulation properties				
RCT, min	11.62	6.45	18.15	32
a_{30}, mm	40.22	22.78	57.68	27
Production traits				
Milk yield, kg/d	8.71	3.20	14.00	40
DIM, d	124	6	299	75
Quality traits				
Fat, %	7.66	5.89	10.10	18
Protein, %	4.56	3.84	5.35	11
Casein, %	3.92	3.21	4.79	12
SCS	3.47	0.87	7.09	57
pH	6.69	6.48	6.91	2

[1]P5 = 5th percentile; P95 = 95th percentile.

[2]RCT = rennet coagulation time; a_{30} = curd firmness after 30 min from rennet addition.

min and it was classified as noncoagulating. In addition to MCP, information on milk yield, fat, protein, and casein contents, pH, and SCC was available. All traits were recorded the same day of sampling, and quality aspects were measured on samples used for MCP analysis. Values of SCC were log-transformed to SCS. Information on buffaloes and herds were provided by the Breeders Associations of Treviso and Padova provinces, and Friuli-Venezia Giulia region.

Sources of variation of RCT and a_{30} were investigated using the GLM procedure (SAS Inst. Inc., Cary, NC). To avoid multicollinearity among explanatory variables, several models accommodating different factors in different steps were fitted as a preliminary analysis. A basic model accounted for the effects of herd, DIM, and parity of cows. Then, effects of milk yield, fat content, protein content, casein content, SCS, and pH were added one at a time, and the increment of the coefficient of determination was used as a criterion for model choice. Finally, the operational model for both RCT and a_{30} considered the effects of herd (5 levels), DIM (5 levels; class 1: <60 d, class 2: 60 to 119 d, class 3: 120 to 179 d, class 4: 180 to 239 d, and class 5: >239 d), parity of cows (4 levels; first, second, third, and fourth or later lactations), fat content (5 levels; class 1: <6.72%, class 2: 6.72 to 7.34%, class 3: 7.35 to 8.18%, class 4: 8.19 to 9.04%, class 5: >9.04%), and pH (4 levels; class 1: <6.59, class 2: 6.59 to 6.67, class 3: 6.68 to 6.79, class 4: >6.79). Besides these factors, the model for a_{30} included the effect of casein content (5 levels; class 1: <3.58%, class 2: 3.58 to 3.74%, class 3: 3.75 to 4.00%, class 4: 4.01 to 4.32%, class 5: >4.32%). Note that all the sources of variation were included as class effects because many nonlinear patterns between response variables and predictors were detected in a preliminary analysis. In addition, for each effect, a multiple comparison of means was performed using the Bonferroni test ($P < 0.05$).

Descriptive statistics of MCP, production, and quality traits are summarized in Table 1. Rennet coagulation time and a_{30} averaged 11.62 min and 40.22 mm, respectively, and showed large variation, with CV of 32% for RCT and 27% for a_{30}. Values of MCP were close to those recommended by Zannoni and Annibaldi (1981) in practical cheese making. Rennet coagulation time was shorter and a_{30} lower than previous studies on

Table 2. Results from ANOVA (*F*-values and significance) for rennet coagulation time (RCT, min) and curd firmness after 30 min from rennet addition (a_{30}, mm)

		RCT, min		a_{30}, mm	
Effect	df	*F*-value	*P*-value	*F*-value	*P*-value
Herd	4	9.11	<0.01	7.34	<0.001
DIM	4	4.50	<0.01	0.35	0.846
Parity	3	1.57	0.193	0.05	0.985
Fat, %	4	2.55	<0.05	0.25	0.908
Casein, %	4	—	—	3.05	<0.05
pH	3	11.73	<0.001	2.97	<0.05
R^2, %		51		48	
RMSE[1]		2.84		8.59	

[1]RMSE = root mean square error.

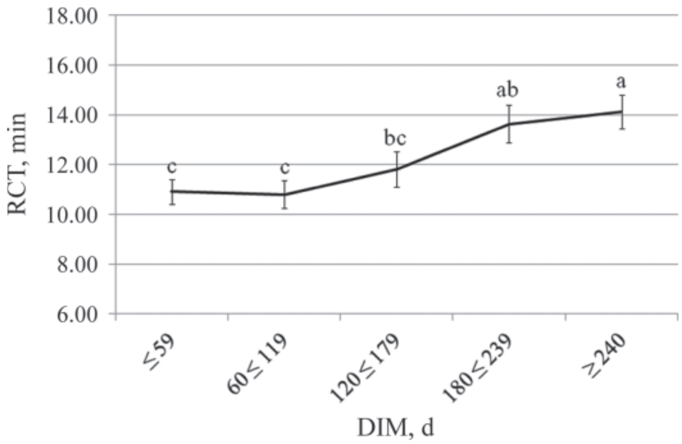

Figure 1. Least squares means of rennet coagulation time (RCT, min) across DIM. Least squares means with different letters are significantly different ($P < 0.05$) according to Bonferroni correction.

individual milk samples from buffalo cows from central and southern regions of Italy (Bartocci et al., 2002; Ariota et al., 2007; Potena et al., 2007a), but they were more favorable than values reported for dairy cattle breeds (Ikonen et al., 2004; Cecchinato et al., 2011). Milk yield, and fat, protein, and casein contents averaged 8.71 kg/d, and 7.66, 4.56, and 3.92%, respectively, with moderate to high variability (CV between 11 and 40%; Table 1). Values for production and protein were comparable to those reported in previous studies on intensive buffalo farming (Rosati and Van Vleck, 2002; Zicarelli, 2004) and to statistics published by the National Breeders Association of Buffalo Species (ANASB, 2010) on herdbook-registered cows. Values for fat were lower than in previous studies (e.g., Rosati and Van Vleck, 2002; Zicarelli, 2004), with the exception of Tiezzi et al. (2009), who found similar results for this trait in 2 herds of northeast Italy. Casein content was very similar to the value (3.86%) reported by Ariota et al. (2007). Somatic cell score and pH averaged 3.47 and 6.69, respectively. The SCS was lower than values found by Bartocci et al. (2002) in Mediterranean buffalo in central Italy and higher than the average value calculated by Cerón-Muñoz et al. (2002) in Murrah buffalo in Brazil, whereas pH was in agreement with previous reports (Bartocci et al., 2002; Ariota et al., 2007).

Results from the ANOVA of RCT and a_{30} are summarized in Table 2. The coefficients of determination of the model were 51 and 48%, respectively, suggesting that factors included in the analysis were able to account for about half of the total variability shown by MCP. The herd effect significantly influenced both RCT ($P < 0.01$) and a_{30} ($P < 0.001$). Herds differed in terms of technological properties (data not shown), probably reflecting differences in management and feeding practices. Potena et al. (2007a) and Bartocci et al. (2002) estimated significant differences among buffalo farms for MCP, according to their level of production, with the best results in high-producing herds. Ojala et al. (2005) reported that herd effects had a markedly lower effect on the variation of MCP than of milk yield traits in dairy cows. Consequently, the differences in feeding and management practices had only a small effect on the variation of technological properties of milk. Tyrisevä et al. (2004) observed that frequent feeding of concentrates was associated with a slight improvement of MCP as well as of milk, fat, and protein yields.

Days in milk affected only RCT ($P < 0.01$; Table 2), and the pattern of LSM for this trait across DIM is depicted in Figure 1. Milk from buffaloes at an early stage of lactation exhibited the most favorable values of RCT, in agreement with Bartocci et al. (2002). The variation of MCP across DIM could be related to changes in physical and chemical characteristics of milk during lactation, with regard to its basic component, micelle structure, and salt equilibrium and, consequently, its technological and physicochemical properties. Thus, these changes can affect the yield and quality of the resulting product such as in Cheddar (Kefford et al., 1995) and Mozzarella cheese (Kindstedt and Fox, 1993). Parity of cows did not affect ($P > 0.05$; Table 2) MCP, confirming findings from previous studies on dairy cows (Lindström et al., 1984; Davoli et al., 1990).

Fat content was an important source of variation for RCT ($P < 0.05$; Table 2), but not for a_{30} ($P = 0.908$). The lack of association between fat and curd firmness was previously reported by Aleandri et al. (1989) on dairy cows. Figure 2 shows that RCT increased with

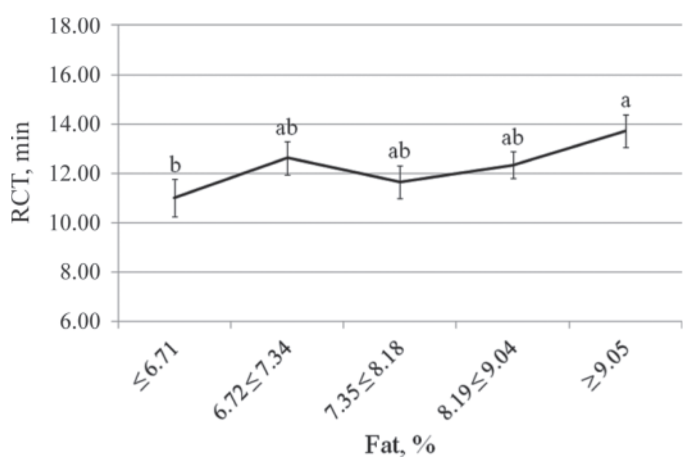

Figure 2. Least squares means of rennet coagulation time (RCT, min) across classes of fat content. Least squares means with different letters are significantly different ($P < 0.05$) according to Bonferroni correction.

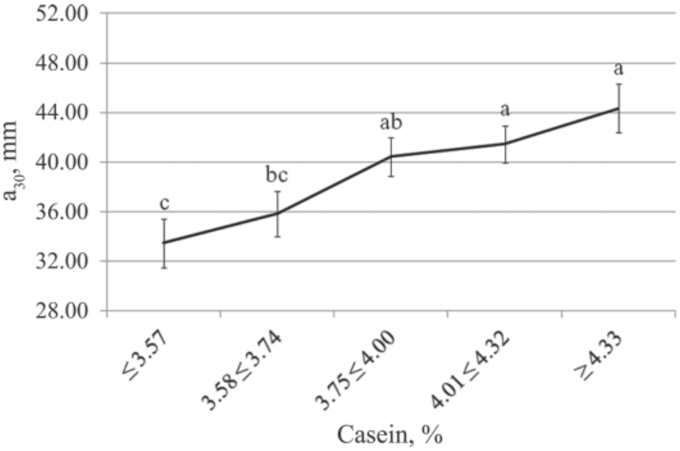

Figure 3. Least squares means of curd firmness after 30 min from rennet addition (a_{30}, mm) across classes of casein content. Least squares means with different letters are significantly different ($P < 0.05$) according to Bonferroni correction.

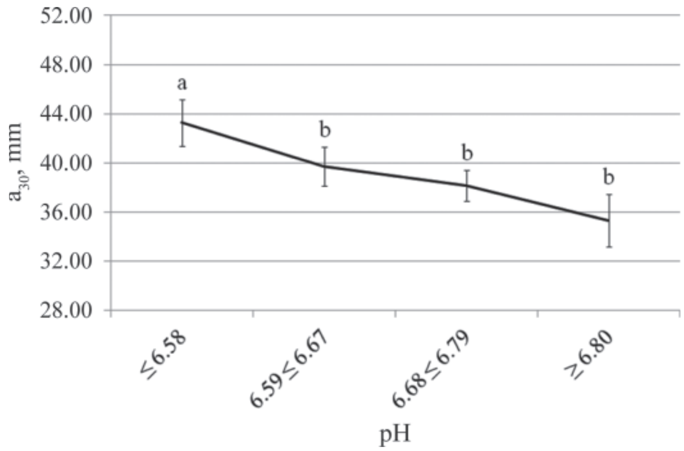

Figure 5. Least squares means of curd firmness after 30 min from rennet addition (a_{30}, mm) across milk acidity. Least squares means with different letters are significantly different ($P < 0.05$) according to Bonferroni correction.

fat content and the multiple comparison of means highlighted a significant difference ($P < 0.05$) between the 2 extreme classes of fat percentage. Casein had an important role in explaining the variability of a_{30} ($P < 0.05$; Table 2). The pattern of LSM of a_{30} across classes of casein content is depicted in Figure 3. Buffaloes that produced milk with a high casein percentage showed the most favorable values of a_{30}. Curd firmness influences both the yield and quality of cheese (Aleandri et al., 1989; Ng-Kwai-Hang et al., 1989) and is significantly affected by the relative proportions of different caseins and casein genotypes (Pagnacco and Caroli, 1987; Bonfatti et al., 2010). Aggregated caseins form cross-linked network structures that entrap fat globules and some whey solids during cheese manufacture. A high casein content in milk will entrap more fat and whey solids in curd; thus, cheese yield will be increased.

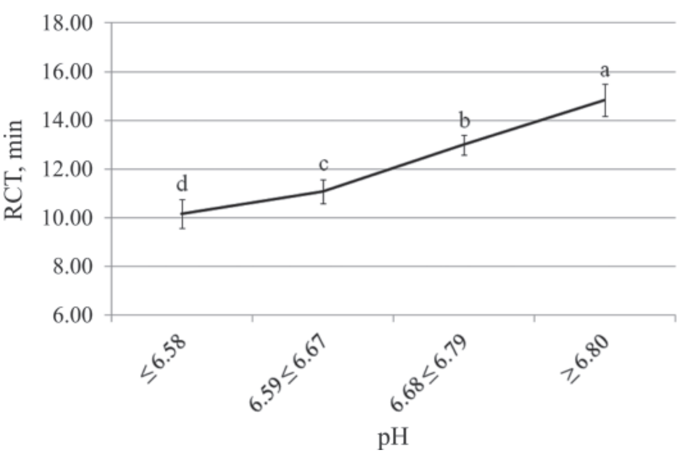

Figure 4. Least squares means of rennet coagulation time (RCT, min) across classes of milk acidity. Least squares means with different letters are significantly different ($P < 0.05$) according to Bonferroni correction.

The acidity of milk largely influenced the variation of RCT ($P < 0.001$) and a_{30} ($P < 0.05$; Table 2), confirming findings from Potena et al. (2007a). The pattern of LSM of the 2 traits across classes of pH showed that milk with high pH exhibited longer RCT (Figure 4) and lower a_{30} (Figure 5) than milk with low pH. Okigbo et al. (1985) reported that curd firmness of milk from dairy cows decreased when pH increased and, generally, milk samples did not coagulate when pH was >6.85. Other authors (Ikonen et al., 2004) confirmed that pH exerts significant effects on curd firmness. Changes in pH are known to affect enzyme activity (Okigbo et al., 1985) and plasmin is less active in low pH milk (Nielsen, 2003). Because associations among SCS, pH, and plasmin activity exist, recursive relations between these traits, which were not possible to disentangle in this study, can be envisaged (Cecchinato et al., 2011).

In conclusion, several factors affected MCP of buffalo cows. Rennet coagulation time and a_{30} were influenced by herd and pH, along with DIM and fat content for RCT, and casein content for a_{30}. Although additional research is needed to investigate the effect of these sources of variation on cheese yield, casein content and acidity may be used as indicator traits to improve the technological properties of buffalo milk.

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