Ca and P in buffalo milk: curd yield and milk clotting parameters

B. Ariota¹, G. Campanile¹, A. Potena², R. Napolano², B. Gasparrini¹, G. L. Neglia¹, R. Di Palo¹

¹ Dipartimento di Scienze Zootecniche e Ispezione degli alimenti. Università di Napoli Federico II, Italy
² Associazione Nazionale Allevatori Specie Bufalina. Caserta, Italy

Corresponding author: Barbara Ariota. Dipartimento di Scienze Zootecniche e Ispezione degli alimenti. Facoltà di Medicina Veterinaria, Università di Napoli Federico II. Via F. Delpino, 1, 80137 Napoli, Italy - Tel. +39 081 2536073 - Fax: +39 081 292981 - Email: barbara@ariota.it

ABSTRACT: Aim of this study was to evaluate the mineral milk content and its relationship with the cheese yield and the rennet coagulation properties. Ca and P content, total and soluble, was determined on 70 milk samples along with the physic-chemical composition, cheese yield and coagulation parameters. Total Ca and P contents were 170.57±14.41 mg·dl⁻¹ and 145.34±26.87 mg·dl⁻¹, with a Ca/P ratio of about 1.2. Fresh cheese yield was on average 261.7±25.4 gr·l⁻¹ of milk and was influenced by both milk quality and Ca and P contents (R²=0.82). The average rennet coagulation parameters had the following values: R=14.20±3.82; K20=1.73±0.97 and A30=46.01±8.81. R values was influenced positively (R²=0.68) by milk pH, protein and fat content and negatively by the Ca/P ratio while shorter K20 value were linked to low micellar Ca and higher soluble P (R²=0.46). The A30 was negatively influenced by milk pH, fat/protein ratio and positively by soluble Ca and P content and micellar P % (R²=0.50).

Key words: Calcium, Phosphorus, Cheese yield, Buffalo.

INTRODUCTION – Nowadays, the genetic merit of buffalo species is referred to PKM index that is based on the evaluation of the kg of mozzarella production per lactation assessed by using an algorithm that includes only the milk protein and fat percentage. Recent studies (Zicarelli, 2004) showed that individual milk samples had different cheese yield even if they did not differ in the chemical composition. It is well known that different coagulation time and rennet coagulation properties come from differences in milk composition linked to other factors than merely fat and protein. The aim of this study was to evaluate the content and the role of the main mineral milk salts, Ca and P, and their relationship with the cheese yield and the rennet coagulation properties.

MATERIAL AND METHODS – Individual milk samples were collected at the complete morning milking from 70 buffalo in three farms at an average of 156±46 days in milk. A complete chemical composition (ASPA, 1995), pH, lattodinamographic parameters (Formagraph), the cheese yield (Intrieri et al. 1986) at 1 hour (fresh), after 28 h and its dry matter, total and soluble P (colorimetric methods), total and soluble Ca (Atomic absorption spectrophotometry) were determined for each sample. The soluble fraction of milk salts were determined on the serum obtained by rennet coagulation (ASPA, 1995). The micellar salt contents were obtained by difference. Somatic cells (SSC) were counted by a Fossomatic cell counter and total aerobic mesophilic flora (TAMF) was determined by the dilution method. Correlation and regression analyses with stepwise method were performed for cheese yield and coagulation parameters in relation to the physic-chemical ad microbiological milk composition.

RESULTS AND CONCLUSIONS – The average calcium content was slightly lower than those found in the past in Italian Buffalo milk (Ferrara and Intrieri, 1974) and similar to Ca content reported by Kon and Cowie (1971). Besides, total P milk content (Table 2) was slightly higher than in the past (Ferrara and Intrieri, 1974), due to an increase in the soluble fractions. The micellar Ca per casein unit was 30.0±5.1 mg·dl⁻¹ that in terms of molar concentration was 0.75±0.12 mM, slightly lower to what found in cow milk (Gaucheron, 2005), while the Ca/P ratio was about the same reported by Summer et al. (2002) for the Modenese cow and lower than that reported for Friesian cow. No correlations were found between mineral content and number of days in milk.
Fresh cheese yield was on average 261.7 ± 25.4 gr·l⁻¹ of milk and lowered to 252.8 ± 38.1 gr·l⁻¹ of milk after 28 h, with a mean reduction of about 3.4%. Regression analysis performed on fresh cheese yield data (gr·l⁻¹ of milk) showed an influence of both milk quality and Ca and P contents ($R^2=0.89$):

$$\text{Fresh cheese yield} = -130.0 + 60.8 \text{ (protein %)} + 6.5 \text{ (fat %)} + 0.31 \text{ (soluble P mg·dl}^{-1} \text{)} + 0.56 \text{ (micellar Ca%)} - 0.15 \text{ (urea mg·dl}^{-1} \text{)}.$$  
Cheese yield after 28 h was influenced more by milk P content with a higher reliability ($R^2=0.93$):

$$\text{Cheese yield 28 h} = 356.70 + 56.22 \text{ (protein %)} + 5.04 \text{ (fat %)} + 0.22 \text{ (total P mg·dl}^{-1} \text{)} - 1.10 \text{ (urea mg·dl}^{-1} \text{)} - 59.88 \text{ (pH)}.$$  
Dry matter (DM) cheese yield was influenced by the better milk quality and by milk P as follow ($R^2=0.85$):

$$\text{DM cheese yield:} 161.42 + 20.09 \text{ (protein %)} + 4.79 \text{ (fat %)} - 0.11 \text{ (total P mg·dl}^{-1} \text{)} + 0.04 \text{ (days in milk)} - 25.70 \text{ (pH)}.$$  
The positive effects of high fat and protein contents are well documented in many studies so as an improvement in the cheese yield in milk with higher content of micellar Ca (Mariani et al., 1997; Mariani and Battistotti 1999). In our data milk P content seems to have greater relevance to favor cheese yield, especially in relation to the curd hydration. The average rennet coagulation parameters had the following values: $R = 14.20 ± 3.82; K_{20} = 1.73 ± 0.97$ and $A_{30} = 46.01 ± 8.81$, quite different from those found in cow (Mariani et al., 1997; Summer et al., 2002; Summer et al., 2004), with the time of the entire phase of coagulation being lower and the curd firmness higher in buffalo than in cow. Correlation analyses showed that higher time in both coagulation phases determined lower curd firmness ($r = −0.38$ and $r = −0.72$ for R and $K_{20}$ respectively; $P < 0.01$) and that higher R values were linked to higher cheese yield ($r = 0.43$ with fresh cheese yield; $P < 0.01$ and $r = 0.28$; $P < 0.05$ for 28 h cheese yield). The time R was influenced positively ($R^2=0.68$) by milk pH, protein and fat content but also negatively by the Ca/P ratio. The importance of the pH on the rennet coagulation time is well known (Mariani and Battistotti, 1999). A lower Ca/P ratio, especially linked to a low value of micellar Ca (Mariani and Battistotti, 1999) and of soluble Ca (Salvadori del Prato, 1987), is reported to be of relevance to the curd formation phase with a reflex also on the curd firmness. Besides, a higher influence on R value of an increase in milk P than a decrease in Ca content was found in our data that needs to be confirmed by further studies. The curd firming time was influenced only by the milk mineral balance ($R^2=0.46$) with shorter $K_{20}$ value linked to lower micellar Ca and higher soluble P content. It is worth noting that this parameter was very low compared to the average value reported in cow of different breed (Summer et al., 2002; Summer et al., 2004) but it agrees with what found in other research on buffalo milk (Adddeo et al., 1995; Esposito et al., 1997). This may depend on the tendency to faster aggregation of the casein micelles in milk with high casein content (Fossa et al., 1994) like that in buffalo milk; another explanation may be the higher k-casein content of buffalo milk (Adddeo et al., 1995). $A_{30}$ in the regression analysis ($R^2=0.50$) was positively influenced by micellar P percentage, soluble Ca and P content while milk pH and fat/protein ratio showed negative relationships. An increase in the soluble Ca and

---

**Table 1.** Mean, sd, minimum (Min) and maximum (Max) values of chemical components, somatic cell (SSC) and total aerobic mesophilic flora (TAMF) in buffalo milk.

| Component | Fat | Protein | Casein | Ash | pH (log 10) | SSC (log 10) | TAMF |
|-----------|-----|---------|--------|-----|------------|-------------|------|
| Mean      | 8.71| 4.71    | 3.86   | 0.83| 6.58       | 4.98        | 5.41 |
| sd        | 1.38| 0.26    | 0.29   | 0.04| 0.07       | 0.37        | 0.74 |
| Min       | 5.00| 3.94    | 2.79   | 0.71| 6.44       | 4.20        | 1.66 |
| Max       | 13.00| 5.37   | 4.51   | 0.91| 6.78       | 5.73        | 6.30 |

**Table 2.** Mean, sd, minimum (Min) and maximum (Max) values of the different Ca and P fractions in buffalo milk.

| Fraction     | Total Ca | Soluble Ca | Micellar Ca | Total P | Soluble P | Micellar P | Ca/P ratio |
|--------------|----------|------------|-------------|---------|-----------|------------|------------|
| Mean         | 170.57   | 55.21      | 115.36      | 145.34  | 56.87     | 88.47      | 1.21       |
| sd           | 14.41    | 10.88      | 17.79       | 26.87   | 12.92     | 20.35      | 0.20       |
| Min          | 150.04   | 38.36      | 74.07       | 104.00  | 30.00     | 50.00      | 0.85       |
| Max          | 205.91   | 96.93      | 166.45      | 196.73  | 87.23     | 128.86     | 1.62       |
P, as reported above, improve the curd firmness also in cow while the higher fat/protein ratio is reported to be responsible for improved elastic property in buffalo milk than in cow (Addeo et al., 1995). Our data seem to show that a too high fat content, not related to an optimal protein rate, could produce the formation of structurally defective curd mass. The relationships between cheese yield and milk salts in buffalo seem to agree with what found in the cow. However, it should be better clarified the role of P that seemed to be more relevant than that of Ca. Both mineral salts were important to define the rennet coagulation properties of buffalo milk as in cow, even if with a relevant importance of the soluble fraction.

The research was supported by C.R.A.A. (Campania Region - Italy).

**REFERENCES**

– Addeo F., Emaldi G.C, Masi P., 1995. Tradizione e innovazione nella produzione della “Mozzarella di bufala Campana” Bubalus Bubalis. III: 46-62. ASPA, 1995. Analisi del latte delle principali specie di interesse zootecnico. Centro Stampa Università di Perugia, Italia. Esposito L., Di Palo R., De Barros Pinto H.M., Ricci G, Zicarelli L., 1997. Variations in lactodinamometric characteristics of Mediterranean Buffalo milk from individual animals. Proc. 5th World Buffalo Congress, 225-230. Ferrara B., Intrieri F., 1974. Caratteristiche ed impiego del latte di bufala. Zoot. Vet., 1-2:14-125. Fossa E., Pecorari M., Sandri S., Tosi F., Mariani P., 1994. The role of milk casein content in the parmigiano-reggiano cheese production: chemical composition, rennet coagulation properties and dairy technological behaviour of milk. Scienza e tecnica lattiero casearia 45,(6):519-535. Gaucheron F., 2005. The minerals of milk. Rev. Reprod. Nutr. Dev. 45: 473-483. Intrieri F., Barbieri V., de Francis G., Cavalieri A. e Altiero V., 1986. Proc.XI Conv. SISVet., 754-756. Kon S.K., Cowie A.T., 1971. Milk vol 2°, Acad. Press, New York. Mariani P. Battistotti B., 1999. Milk quality for cheesemaking. Proc. XIII A.S.P.A. Congress, 499-516. Mariani P., Serventi P., Fossa E., 1997. Contenuto di caseina, varianti genetiche ed attitudine tecnologico-casearia del latte delle vacche di razza bruna nella produzione del formaggio grana. La razza bruna italiana 2:8-14. Salvatori Del Prato O., 1998. Trattato di tecnologia casearia, Ed. Edagricole, Bologna. SPSS. 2005. User’s Guide. Version 12.0. SPSS Inc. Chicago, IL, USA. Summer A., Malacarne M., Martuzzi F., Mariani P., 2002. Structural and functional characteristics of Modenese cow milk in Parmigiano-Reggiano cheese production. Ann. Fac. Medic.Vet. di Parma, XXII :163-174. Summer A., Pecorari M., Fossa E., Malacarne M., Formaggioni P., Franceschi P., Mariani P., 2004. Frazioni proteiche, caratteristiche di coagulazione presamica e resa in formaggio Parmigiano-Reggiano del latte delle vacche di razza Bruna Italiana. Proc. 7a Conferenza Mondiale Allevatori Razz Bruna, 77-82. Zicarelli L., 2004. Buffalo milk: its properties, dairy yield and mozzarella production. Vet. Res. Com. 28:127-135.