The Effects of the Somatic Cell Count on Yield, Composition and Coagulating Properties of Mediterranean Buffalo Milk**

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ABSTRACT : The monitoring was carried out for one year on 20 farms of Mediterranean buffalo situated in central Italy. The milk yield, the somatic cell count, the coagulating properties and some components were determined. The average value of somatic cells was 221.28 n×10^3/ml. Milk production decreased when somatic cell numbers increased. The rennet clotting time increased significantly when somatic cells were higher than 300.00 n×10^3/ml, the curd firming time was significantly higher when somatic cells were more than 1,000.00 n×10^3/ml and the curd firmness increased up to 200.00 n×10^3/ml, then gradually decreased. Protein and casein decreased when somatic cells increased and the same trend was shown by casein/protein ratio. Both for these components and the coagulating properties the threshold limit of somatic cells to obtain better results was 200.00 n×10^3/ml. The somatic cell number did not show a trend which was strictly influenced by the lactation stage, contrary to what happened in the other species. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 5 : 738-742)***

Key Words : Buffalo Milk, Somatic Cells, Milk Yield, Milk Composition, Coagulating Properties

INTRODUCTION

A consistent number of buffaloes is at present registered in three European countries: Italy, Bulgaria and Romania and in recent years the buffalo population has been increasing in Italy. Almost all buffalo milk is assigned to cheese making, mainly to "Mozzarella", a typical Italian cheese; therefore it is important to achieve milk which is able to produce high quantity and good quality cheese.

Somatic cell count is usually utilised as a sanitary control of milk and specially as an indicator of sub-clinical presence of mastitis. Inflammation of mammary epithelium has the following effects on milk production: reduced synthesis of milk components synthesised by tissue cells (fat, casein and lactose), increased passage of blood component to milk (albumin and immunoglobulin) and a reduced milk yield (Anderson and Andrews, 1977; Verdi et al., 1987; Kalorey et al., 2001). The alteration of the milk composition affects cheesemaking properties and cheese yield and composition. Some studies have been carried out on cow milk (Politis and Ng-Kwai-Hang, 1988abc; Auldist et al., 1996) but little is known about buffalo milk (Esposito et al., 1997).

The aim of this paper is to study the effect of somatic cells on yield, composition and cheesemaking properties of milk from Mediterranean buffalo bred in Italy.

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MATERIALS AND METHODS

The monitoring was carried out on 20 farms of Mediterranean buffalo. The number of controlled milking buffaloes on each farm was proportional both to total number of animals and standard deviation of average daily milk yield of the previous year. For one year the monthly milk yield of controlled buffaloes was measured and 1,859 milk samples were collected, added to Bronopol and analysed the day after milking.

The somatic cell count by fluoro-opto-electronic method (Somacounter 300, Bentley Instruments, U.S.A.) and coagulating properties (r=rennet clotting time, K_{20}=curd firming time, A_{30}=curd firmness) by Formagraph (Foss Electric, DK) according to the Zannoni and Annibaldi method (1981) at 35°C using a liquid rennet (C. Hansen), 1:15.000 rennet units, 90% of chymosin, diluted 1:100 and added to whole milk as 200 μl/10 ml were determined on all milk samples.

The protein content by Kjeldahl and casein by Rowland (1938) modified by Ashaffenburg and Drewry (1959) were determined on 5% of the samples (one sampling on about 1/3 of buffaloes in the middle lactation).

The milk samples were divided into classes according to somatic cell number and lactation days, and the statistical differences among classes were found by analysis of variance using a monofactorial model by SAS/GLM procedure; furthermore a regression equation between daily milk yield and somatic cell count was obtained using SAS/REG procedure (SAS, 1993).

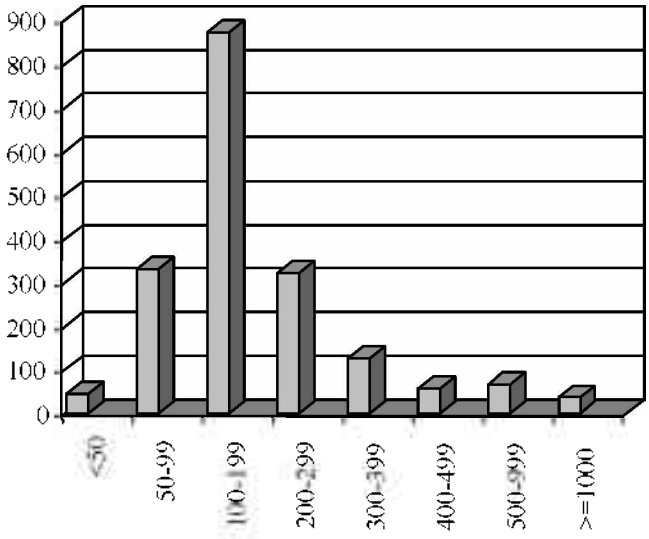

Figure 1. Number of mediterranean buffalo milk samples in the somatic cell classes (n×10^3/ml)

RESULTS AND DISCUSSION

The somatic cell number of a high percentage of samples was included between 50.00 to 300.00 n×10^3/ml (figure 1) and the average value was 221.28 n×10^3/ml (table 1).

According to Esposito et al. (1997) the somatic cell average value of Mediterranean buffaloes is lower, 169.00 n×10^3/ml, and was the result of some samples coming from only one farm while the data from Tantillo et al. (1997) are higher (309.00 n×10^3/ml) and they always came from only one farm. The average value of somatic cell count observed on 37 Italian farms from 1997 to 2000 was 191.81 n×10^3/ml (S. Terramoccia et al., unpubl. data). The somatic cell number observed in Surti, Murrah and Lankan buffaloes varied from 50.00 to 375.00 n×10^3/ml and on the average 140.00 n×10^3/ml (Silva and Silva, 1994).

If one examines that the limit allowed by European legislation on cow milk is 400.000 somatic cells/ml and that this limit reflects the actual sanitary status of cow milk, one can conclude that the average somatic cell number of buffalo milk is lower than that of cow milk. In the buffalo species the very low frequency of mastitis was observed and one of the motives which favours buffalo breeding above all in Asia and Middle East is its elevated resistance to mastitis (Wanasinghe, 1985).

Table 1. Health characteristics, daily yield, acidity and coagulating properties of Mediterranean buffalo milk

	Mean±SD
Somatic cells (n×10^3/ml)	221.28±297.07
Yield milk (kg/d)	9.33±3.72
pH	6.72±0.11
Rennet clotting time, r (min)	14.87±4.70
Curd firming time, K_{20} (min)	3.24±1.33
Curd firmness, A_{30} (mm)	48.04±12.36

In table 1 the average values of milk yield, pH and coagulating properties are also reported. The milk yield was lower than that observed by Verna et al. (1993ab) during an experimental trial carried out on first 5-6 months of lactation (9.33 vs 10.71 kg/d). The average pH value, 6.72, was similar to that observed by Tripaldi et al. (1997) in the above mentioned trial. The coagulating properties (r=14.87 min; K_{20}=3.24 min; A_{30}=48.04 mm) were different from those noted by the same Authors who observed lower rennet clotting time (r=10.80 min) and curd firming time (K_{20}=2.62 min) but higher curd firmness (A_{30}=57.75 mm), in other words better cheesemaking properties.

In table 2 the average values of milk yield, pH and coagulating properties according to different somatic cells classes are reported.

Milk production decreased from 9.32 to 7.99 kg/d when the somatic cell number increased and the same trend was reported in cow milk (Ng-Kwai-Hang et al., 1984). Gill and Holmes (1978) calculated a significative linear equation between somatic cell numbers and daily milk yield on dairy cow bulk milk and with data from table 2 the following equation was obtained: Y=9.8999-0.0029X (R^2=0.92, p<0.01), where X=somatic cell number (n×10^3/ml) and Y= daily milk yield (kg/d).

The milk pH increased significantly, from 6.74 to 6.80, when the somatic cells increased from 300.00-399.00 to 500.00-1,000.00 n×10^3/ml. A higher increase in sheep milk was observed by Duranti and Casoli (1991), where the pH increased from 6.62 to 6.71 when the somatic cells went from 300.00-500.00 to 500.00-1,000.00 n×10^3/ml. According to Muelas et al. (1994), who analysed sheep milk,

Table 2. Estimated means of daily yield, acidity and coagulating properties of mediterranean buffalo milk according to the somatic cell number

Somatic cells (n*10^3/ml)	<50	50-99	100-199	200-299	300-399	400-499	500-999	≥1000	Rmse
Yield milk (kg/d)	9.32^{ab}	9.93^{a}	9.55^{ab}	8.96^{abc}	8.62^{bc}	8.61^{bc}	7.89^{c}	7.99^{c}	3.70
pH	6.73^{de}	6.71^{de}	6.70^{d}	6.72^{de}	6.74^{bc}	6.77^{ab}	6.80^{a}	6.79^{a}	0.11
Rennet clotting time, r (min)	14.64^{b}	14.27^{b}	14.53^{b}	14.73^{b}	16.68^{a}	16.93^{a}	17.42^{a}	17.03^{a}	4.63
Curd firming time, K_{20} (min)	3.57^{bcd}	3.45^{cde}	3.05^{e}	3.12^{de}	3.48^{cde}	3.95^{ab}	3.66^{bc}	4.20^{a}	1.31
Curd firmness, A_{30} (mm)	42.64^{de}	45.77^{bc}	50.28^{a}	48.37^{ab}	45.17^{bc}	44.25^{de}	43.60^{de}	40.58^{d}	12.12

a, b, c, d, e: p<0.05

Table 3. Estimated means of protein and casein of milk samples

Somatic cells ($n\times10^3$/ml)	<50	50-99	100-199	200-299	300-399	400-499	500-999	≥1000	Rmse	Mean±SD
Protein (%)	4.84^{a}	4.72^{ab}	4.77^{ab}	4.30^{abc}	4.15^{bc}	3.66^{c}	3.64^{c}	3.62^{c}	0.45	4.63±0.50
Casein (%)	3.90^{a}	3.81^{ab}	3.90^{a}	3.26^{bc}	3.07^{c}	2.74^{c}	2.71^{c}	2.69^{c}	0.35	3.75±045
Casein/Protein (%)	80.79^{a}	80.31^{a}	82.12^{a}	79.36^{ab}	77.18^{bc}	74.86^{c}	74.75^{c}	74.31^{c}	1.98	81.14±2.46

a, b, c: $p<0.05$

the pH increase is very low, from 6.63 to 6.64, with somatic cells from 344.00 to 747.00 $n\times10^3$/ml. In Holstein Freisian milk a significant increase of pH (from 6.65 to 6.76) was pointed out when the somatic cells went from 250.00-500.00 to 500.00-1.000.00 $n\times10^3$/ml (Haenlein et al., 1973). Politis and Ng-Kwai-Hang (1988c) observed an increase of 0.02 units in cow milk samples which increased the somatic cell number from 400.00 to 900.00 $n\times10^3$/ml.

From the afore mentioned results one can argue that the milk pH did not change to the same degree when faced with a variation of somatic cell count and it is probably because this trend did not depend on the milk characteristics of each species, but from other causes still difficult to identify.

The rennet clotting time increased significantly (from about 14-15 to about 17 min) when the somatic cell numbers were more than 300.00 $n\times10^3$/ml. In cow milk the increase of rennet clotting time in samples containing 1.000.00 $n\times10^3$/ml of somatic cells was about 21% compared to samples containing 100.00 $n\times10^3$/ml (Politis and Ng-Kwai-Hang, 1988c). The difference between buffalo milk results connected with 1.000.00 and 100.00 $n\times10^3$/ml of somatic cells was about 19%. In sheep milk this parameter duplicated from 9.40 to 18.90 minutes when somatic cells varied from less then 300.00 to more than 1.000.00 $n\times10^3$/ml (Duranti and Casoli, 1991). According to Pirisi et al. (1994) the rennet clotting time of sheep milk was 31.79 and 18.57 minutes respectively for 3.300.00 and 450.00 $n\times10^3$ /ml of somatic cells.

The curd firming time was significantly higher when somatic cells were more than 1.000.00 $n\times10^3$/ml (4.20 min). In cow milk a significant increase was observed in samples with more than 500.00 $n\times10^3$/ml of somatic cells (Politis and Ng-Kwai-Hang, 1988c). The same results were obtained by Duranti and Casoli (1991) in sheep milk.

The curd firmness increased up to 200.00 $n\times10^3$/ml of somatic cells (maximum value=50.28 mm), then gradually decreased. In cow and sheep milk a decrease of firmness was observed when somatic cells were higher than 500.00 $n\times10^3$/ml (Politis and Ng-Kwai-Hang, 1988c; Duranti and Casoli, 1991).

From the examination of the above parameters it is clear that the coagulating properties of milk with a high somatic cell number are not ideal to make good cheese and therefore obtain an adequate cheese yield. According to these results it would be better not to exceed 200.00 $n\times10^3$/ml of somatic cells.

The contents of protein and casein, grouped by somatic cell classes, are reported in table 3. Both components decreased when somatic cells increased (protein: from 4.84 to 3.62 %; casein: from 3.90 to 2.69 %) and the same trend was shown by the casein/protein ratio. It seems that to obtain better values of these parameters 200,000 somatic cells/ml should not be exceeded. The same threshold has been mentioned on the coagulating properties and this is obvious that there is the close relation between these components and coagulating properties observed in buffalo milk (Tripaldi et al., 1997).

According to Coulon et al. (1998) the casein/protein ratio decrease with the presence of higher somatic cell number could be due to many factors: reduced casein synthesis, whey-protein increase and casein degradation caused by the increase of plasmin, an enzym whose content increases in presence of mastitis.

Some Authors found that in cow milk the protein content did not vary significantly when the somatic cells were higher (Haenlein et al., 1973; Ng-Kwai-Hang et al., 1984; Ballou et al., 1995) and the same trend was pointed out in sheep milk by Duranti and Casoli (1991). It was observed that minor casein synthesis was counterbalanced by whey-protein increasing and the protein content did not vary (Haenlein et al., 1973; Ng-Kwai-Hang et al., 1984; Duranti and Casoli, 1991). In buffalo milk it is necessary to verify this by further analyses.

It is interesting to observe (table 4) that somatic cell number in buffalo milk did not have a trend strictly

Table 4. Estimated means of somatic cell number and daily yield during lactation

Lactation days (d)	Milk yield (kg/d)	Somatic cells ($n\times10^3$/ml)
< 30	12.96^{a}	275.77^{a}
30-59	12.52^{a}	228.61^{ab}
60-89	11.83^{b}	203.76^{ab}
90-119	10.36^{c}	212.79^{ab}
120-149	9.19^{d}	198.62^{b}
150-179	7.73^{e}	258.18^{ab}
180-209	6.58^{f}	185.68^{b}
210-239	5.30^{g}	212.48^{ab}
240-269	5.19^{gh}	249.91^{ab}
270-299	4.61^{h}	248.80^{ab}
≥300	4.86^{gh}	227.64^{ab}
Rmse	2.60	298.60

a, b, c, d, e, f, g, h: $p<0.05$

influenced by the lactation stage and then by the milk yield (12.96-4.86 kg/d), even if it varied significantly from one month to an other. Whereas in other kinds of milk it has been observed that somatic cells varied according to the lactation stage: higher values at the beginning, lower between lactation peak and medium and a systematic increase at the end of lactation. For dairy cows, in particular, one can observe a gradual increase of somatic cells between the middle and the end of lactation (Bergonier et al., 1994).

The increase of somatic cells at the end of lactation in healthy animals is due to the concentration of all milk components for a reduced milk yield in this phase; in buffalo, at the end of lactation, the milk yield decreased and the fat and protein increased (Pilla and Moioli, 1993), but the somatic cell count did not increase. It is evident that this aspect needs further study.

In conclusion, the results mainly confirmed that the average somatic cell number in buffalo milk was low and this number did not increase during the second phase of lactation, as in the other species. Therefore when the somatic cell number is above a certain threshold, yet to be defined, there are many probabilities that mastitis is present, therefore the somatic cell count could be a valid indicator of this pathology in buffaloes. This lack of somatic cell increase at the end of lactation makes it necessary to carry out further investigation. Besides, it was observed that in buffalo milk a higher somatic cell count negatively influences some milk components and cheesemaking properties and better results are obtained by not exceeding the threshold of 200.00 $n \times 10^3$/ml of somatic cells.

REFERENCES

Anderson, M. and A. T. Andrews. 1977. Progressive changes in individual milk protein concentrations associated with high somatic cell count. J. Dairy Sci. 44:223-235.

Aschaffemburg, R. and J. Drewry. 1959. New procedure for the routine determination of the various non–casein proteins of milk. In Proceedings of IX International Dairy Congress, pp. 1631-1637

Auldist, M. J., S. Coats, B. J. Sutherland, J. J. Mayes., G. H. Mc Dowele and G. L. Roogers. 1996. Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese. J. Dairy Res. 63:269-280.

Ballou, L. U., M. Pasquini, R. D. Bremel, T. Everson and D. Sommer. 1995. Factors effecting herd milk composition and milk plasmin at four levels of somatic cell counts. J. Dairy Sci. 78: 2186-2195.

Bergonier, D., G. Lagriffoul, X. Bethelot and F. Barillet. 1994. Facteurs de variation non infectieux des comptages de cellules somatiques chez les ovins et caprins laitiers. In International Symposium on "Somatic cells and milk of small ruminants". Sept. 25th-27th, Bella, Italy, pp. 1-20 (session 2).

Coulon, J. B., C. Hurtaud, B. Rémond and R. Vérité. 1998. Facteurs de variation de la proportion de casines dans les protéines du lait de vache. Productions Animales 11:299-310.

Duranti, E. and C. Casoli. 1991. Variazione della composizione azotata e dei parametri lattodinamografici del latte di pecora in funzione del contenuto di cellule somatiche. Zoot. Nutr. Anim. 17: 99-105.

Esposito, L., R. Di Palo, H. M. De Barros Pinto, G. Ricci and L. Zicarelli. 1997. Variations in lactodynamometric characteristics of Mediterranean buffalo milk from individual animals. In Proceedings of 5th World Buffalo Congress, Oct. 13th-16th, Caserta, Italy, pp. 225-230.

Gill, M. S. and C. W. Holmes. 1978. Somatic cell counts, mastitis and milk production in dairy herds. New Zealand J. Dairy Sci. Techn. 13:157-161.

Haenlein, G. F. W., L. H. Schultz and J. P. Zikakis. 1973. Composition of proteins in milk with varying leucocyte contents. J. Dairy Sci. 56:1017-1024.

Kalorey, D. R., N. V. Kurkure, N. K. Nigot, M. P. Patil and V. P. Pathak. 2001. Effect of subclinical mastitis on milk of cross bred SahiwalxJersey cows: a biochemical study. Asian-Aus. J. Anim. Sci. 14:382-383.

Muelas, R., P. Molina, J. R. Diaz and C. Peris. 1994. Relationship between somatic cell count, acidity and California Mastitis test in Manchega ewes milk. In International Symposium on "Somatic cells and milk of small ruminants". Sept. 25th-27th, Bella, Italy, pp.1-6 (session 3).

Ng-Kwai-Hang, K. F., J. F. Hayes,J. E. Moxley and H. G. Monardes. 1984. Variability of test-day milk production and composition and relation of somatic cell counts with yield and compositional changes of bovine milk. J. Dairy Sci. 67:361-366.

Pilla, A. M. and B. M. Moioli. 1993. Factors affecting fat percentage, protein percentage and fat/protein ratio in buffalo milk. In Proceedings of "Prospect of buffalo production in the Mediterranean and the Middle East", EAAP Publication n. 62, pp. 238-241.

Pirisi, A., G. Piredda, F. Podda and S. Pintus. 1994. Effect of somatic cell count on sheep milk composition and cheesemaking properties. In International Symposium on "Somatic cells and milk of small ruminants". Sept. 25th-27th, Bella, Italy, pp.7-12 (session 3).

Politis, I. and K. F. Ng-Kwai-Hang. 1988a. Effects of somatic cell count and milk composition on cheese composition and cheese making efficiency. J. Dairy Sci. 71:1711-1719.

Politis, I. and K. F. Ng-Kwai-Hang. 1988b. Association between somatic cell count of milk and cheese yielding capacity. J. Dairy Sci. 71:1720-1727.

Politis, I. and K. F. Ng-Kwai-Hang. 1988c. Effects of somatic cell counts and milk composition on the coagulating properties of milk. J. Dairy Sci. 71:1740-1746.

Rowland, S. J. 1938. The determination of the nitrogen distribution in milk. J. Dairy Res. 9:42-46.

S.A.S. 1993. User's guide, Statistics, Sas Institute Inc., Cary (NC), USA.

Silva, I. D. and K. F. S. T. Silva. 1994. Total and differential cell counts in buffalo (*Bubalus bubalis*) milk. Buffalo Journal 2: 133-137.

Tantillo, G., A. Vergara and T. Manginelli. 1997. Valutazione degli aspetti igienico-sanitari del latte di bufala. Il Latte 22:70-75.

Tripaldi, C., G. Catillo, F. Martillotti and M. Angelucci. 1997. Influence of some characteristics of diet on milk quality of water buffalo. Buffalo Journal 13:1-13.

Verdi, R. J., D. M. Barbano, M. E. Dellavalle and G. F. Senyk. 1987. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell milks. J. Dairy Sci. 70: 230-242.

Verna, M., S. Bartocci, A. Amici and M. Agostini. 1993a. Effect of diets with different energetic concentration on the qualitative and quantitative yield in lactating buffaloes. In Proceedings of "Prospect of buffalo production in the Mediterranean and the Middle East", EAAP Publication n. 62, pp. 258-261.

Verna, M., S. Bartocci, A. Amici and M. Agostini. 1993b. Prestazioni produttive di bufale in lattazione alimentate con diete a diversa concentrazione energetica e proteica. In Proceedings of 10th ASPA Congress, May 31st-June 3rd, Bologna, Italy, pp. 247-252.

Wanasinghe, D. D. 1985. Mastitis among buffaloes in Sri Lanka. In Proceeding of the 1st World Buffalo Congress, Cairo, Egypt, pp. 1331-1333.

Zannoni, M. and S. Annibaldi. 1981. Standardization of the renneting ability of milk by Formagraph. Sci. Tec. Latt.-Cas. 32: 79-94.