



## Effects of Mastitis on Buffalo Milk Quality

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**ABSTRACT :** The objectives of this study were to compare the effectiveness of different indicators of mammary inflammation in buffalo and to evaluate the association of the indicators with buffalo milk yield, composition, and rennet coagulation properties. This study was carried out at four buffalo farms in central Italy using a total of 50 lactating buffalo. Milk from each buffalo was tested at the beginning, middle, and end of lactation. To evaluate the relationship between mastitis markers and milk components, three classes were defined for each of the following markers: total somatic cell count (TSCC), differential somatic cell count (DSCC), and bacteriological results. The regression coefficient for the reference method and the alternative method of determining TSCC was 0.81, indicating that the method routinely used to analyze buffalo milk consistently underestimated actual TSCC. The milk samples positive for udder-specific bacteria also had higher TSCC values than the samples that were negative for bacteria ( $872 \times 10^3/\text{ml}$  vs.  $191 \times 10^3/\text{ml}$ ). In samples that were positive for udder-specific bacteria, polymorphonuclear leukocytes (PMN) made up greater than 50% of the cells. Moreover, only 1% of the samples in the lowest TSCC class were positive for bacteria. The correlation between TSCC and PMN was stronger (0.70), and PMN values in buffalo milk increased significantly when the TSCC class changed from low (38%) to medium and high (56% and 64%). Milk yield was negatively related to TSCC. Significant changes in lactose (4.87%, 4.80% and 4.64%) and chloride content (0.650 mg/ml, 0.862 mg/ml and 0.882 mg/ml) were also observed with increasing TSCC values. Higher TSCC was associated with impaired rennet coagulation properties: the clotting time increased, while the curd firming time ( $p \leq 0.05$ ) and firmness decreased. We concluded that in buffalo as in dairy cows, TSCC is a valid indicator of udder inflammation; we also confirmed that a value of  $200 \times 10^3$  cells/ml should be used as the threshold value for early identification of an animal affected by subclinical mastitis. In addition to its association with significantly decreased milk yield, a TSCC value above this threshold value was associated with changes in milk composition and coagulating properties. (**Key Words :** Buffalo Milk, Somatic Cells, Polymorphonuclear Leukocytes, Milk Composition, Coagulating Properties)

## INTRODUCTION

Mammary gland inflammation affects milk yield and quality and can lead to great economic losses for dairy farmers and cheese makers. In fact only milk from a healthy udder produces milk of a physiologically normal composition (Hamann, 2002). In mastitis milk, the changes in composition impair coagulation, cheese yield, and composition; some composition changes lead to poor quality cheese and elevated SCC were associated with the production of a cheese with high moisture content (Munro et al., 1984; Grandison and Ford, 1986; Politis and Ng-Kwai-Hang, 1988a; Politis and Ng-Kwai-Hang, 1988b;

Politis and Ng-Kwai-Hang, 1988c; Barbano et al., 1991).

The effects of mastitis on milk yield and milk quality in cows have also been observed for buffalo milk (Sing and Ludri, 2001; Ceron-Munoz et al., 2002; Pasquini et al., 2003; Tripaldi et al., 2003; Singh and Bansal, 2004). Somatic cell count (SCC) is a measure that is widely used to assess mammary health (Smith, 2002). Milk SCC includes all types of cells: polymorphonuclear leukocytes (PMN), macrophages and lymphocytes. An increase in SCC is due largely to an increase in PMN. The primary function of PMN is ingestion and destruction of invading microorganisms, as well as secretion of inflammatory regulators (Kelly et al., 2000). Some researchers view the PMN count as an earlier and more specific indicator than SCC (Kitchen, 1981; O'Sullivan et al., 1992). Differential cell count results in buffalo milk have differed from findings in cow milk (Silva and Silva, 1994; Dhakal, 2004; Thomas et al., 2004; Piccinini et al., 2006); this prompted us to perform additional studies to compare the

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effectiveness of different measures of mammary inflammation in buffalo. A second aim of this paper was to evaluate the association of these indicators with buffalo milk yield, composition, and rennet coagulation properties.

## MATERIALS AND METHODS

This study was carried out at four buffalo farms in different areas of the Latium region of Italy using animals reared using a loose housing system. We used a total of 50 lactating buffaloes for the study and tested milk from each buffalo in the study on three occasions: at lactation days 30±9, 113±9, and 174±9. During the morning milking, the milk yield was recorded and a milk sample was collected from each buffalo. The following analyses were performed: pH determination; fat, protein, and lactose analysis using a FTIR spectrometer; casein analysis using the ISO 17997-2:2004 IDF 29-2 method; chloride analysis using an automatic titrator; and coagulation property analysis (r = rennet clotting time, K20 = curd firming time, A30 = curd firmness at 30 min, A2r = curd firmness at 2r) using the method of Zannoni and Annibaldi (1981). Bacterial count was determined using the fluoro-opto-electronic flow cell method; total SCC (TSCC) was determined using a reference method (ISO 13366-1:2008 IDF 148-1:2008) as well as using an alternative method with a fluoro-opto-electronic flow cell (ISO 13366-2:2006 IDF 148-2:2006). Differential somatic cell count (DSCC) was determined using the May-Grunwald-Giemsa method (Piccinini et al., 2006), and mastitis pathogens were identified using the method described by Rosati et al. (2005). Both TSCC (using the reference method) and DSCC were determined by two laboratories, and the mean of the two values was used for analysis.

Regression analysis between TSCC values obtained using the reference and alternative TSCC methods was performed using the REG procedure, and the correlation between PMN and TSCC (determined using both reference and alternative methods) was performed using the CORR procedure (SAS, 2006). All other data analyses and calculations were based solely on values obtained using the alternative TSCC method. To evaluate the relationship between the presence of mastitis bacteria, TSCC values, PMN values and other milk components, three classes of the presence of mastitis bacteria, TSCC values and PMN values were defined. Data were analyzed by the GLM procedure (SAS, 2006) according to the following model:

$$Y_{ijkl} = \mu + A_i + Pr_j + SCC_k + bX_{ijkl} + E_{ijkl}$$

Where:

$Y_{ijkl}$  = experimental item

$\mu$  = overall mean

$A_i$  = fixed effect of farm ( $i = 1, \dots, 4$ )

$Pr_j$  = fixed effect of milk analysis ( $j = 1, \dots, 3$ )

$SCC_k$  = fixed effect of classes of SCC or PMN or pathogen presence ( $k = 1, \dots, 3$ )

$bX_{ijkl}$  = regression coefficient of  $X_{ijkl}$  on lactation days at each milk analysis

$E_{ijkl}$  = random error of experimental item

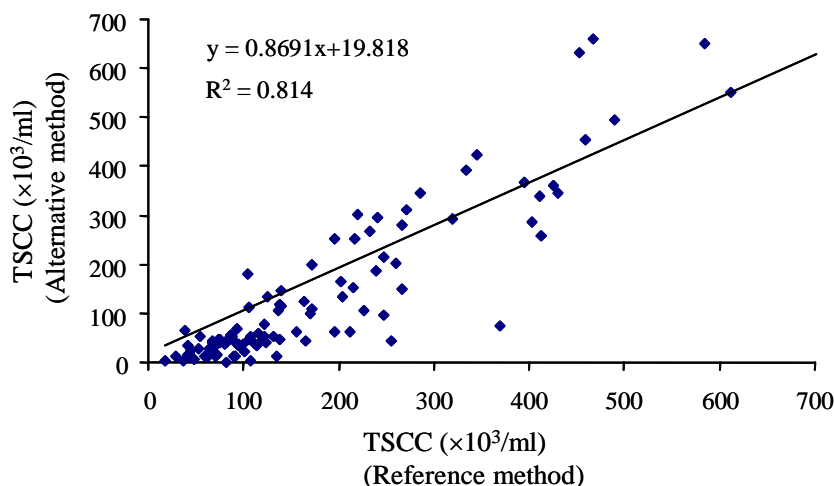
## RESULTS

Table 1 shows the mean values for the components analyzed in buffalo milk. The TSCC results from the reference and alternative methods were  $314 \times 10^3/\text{ml}$  and  $259 \times 10^3/\text{ml}$ , respectively. The regression coefficient for the two methods was 0.81 ( $p < 0.0001$ ) (Figure 1). These data confirmed the results of Orlandini et al. (2009), who determined that the method of estimating TSCC that was routinely used to analyze buffalo milk consistently underestimated actual TSCC, and the underestimation did not correlate with high protein and fat content in buffalo milk.

Bartocci et al. (2002) previously reported a similar TSCC value,  $220 \times 10^3/\text{ml}$ , in animals without clinical symptoms in their udders that were raised the same way as the animals in the present study. Regarding the DSCC,

**Table 1.** Mean values, standard deviation (SD) and ranges for milk yield, milk components and coagulation properties in buffalo milk samples

Item	Mean±SD	Range
Milk yield (L/morning milking)	5.37±2.0	0.5-11.0
TSCC ( $\times 10^3/\text{ml}$ ) (alternative method)	259±666	1-4,651
TSCC ( $\times 10^3/\text{ml}$ ) (reference method)	314±717	17-6,982
PMN (%)	49±20	8-98
Lymphocytes (%)	38±18	1-84
Macrophages (%)	13±11	1-71
Bacterial count (cfu $\times 10^3/\text{ml}$ )	409±646	3-3,783
Fat (%)	7.47±1.54	4.22-12.89
Protein (%)	4.50±0.42	3.44-6.10
Casein (%)	3.62±0.43	1.43-4.91
Lactose (%)	4.78±0.31	3.12-5.34
Chloride (mg/ml)	0.703±0.265	0.228-1.857
pH	6.67±0.10	6.44-6.97
r (min)	20.64±6.01	9.00-57.15
k20 (min)	2.26±1.25	1.45-9.15
a30 (mm)	41.49±10.85	0.00-59.52
a2r (mm)	50.16±7.83	30.00-65.88



**Figure 1.** Regression plot showing the relationship between the two TSCC methods.

PMN were the most prevalent cells (49%), followed by lymphocytes (38%) and macrophages (13%); there was a wide range for each of these cell types. These results agree with those reported by Piccinini et al. (2006) for buffalo milk.

The bacteriological results showed that 9% of the samples were positive for udder-specific bacteria and 13% were positive for environmental bacteria. Table 2 shows that the milk samples positive for udder-specific bacteria also had higher TSCC values than the samples that were negative for bacteria ( $872 \times 10^3/\text{ml}$  vs.  $191 \times 10^3/\text{ml}$ ;  $p < 0.05$ ). This is in accordance with results of other studies (Dhakal,

2004; Thomas et al., 2004).

In the DSCC, PMN made up greater than 50% of the cells only in samples that were positive for udder-specific bacteria; there were no significant differences relative to the other two groups (positive for environmental bacteria and negative for bacteria). Thomas et al. (2004) observed that PMN increased according to the degree of bacterial positivity, but they also observed that the range of PMN in each class of California Mastitis Test (CMT) scores (from negative reaction to strong positive reaction) was very wide, varying from 0-96%.

In samples that were negative for bacteria, the lactose

**Table 2.** Estimated mean values for yield, components and coagulation properties of buffalo milk according to bacteriological results

Bacteriological results	Positive for udder-specific bacteria	Positive for environmental bacteria	Negative for bacteria
% samples	9	13	78
Milk yield (L/morning milking)	5.44±0.60 ns	4.56±0.47	5.50±0.18
TSCC (x10 <sup>3</sup> /ml)	872±215 <sup>a</sup>	305±170 <sup>ab</sup>	191±64 <sup>b</sup>
PMN (%)	54±6 ns	38±7	47±2
Lymphocytes (%)	32±5 ns	48±6	39±8
Macrophages (%)	14±4 ns	14±5	14±1
Bacterial count (cfu x10 <sup>3</sup> /ml)	647±211 ns	660±166	453±63
Fat (%)	7.89±0.47 ns	7.16±0.37	7.45±0.14
Protein (%)	4.44±0.14 ns	4.47±0.11	4.52±0.04
Casein (%)	3.43±0.16 ns	3.65±0.15	3.65±0.05
Lactose (%)	4.46±0.09 <sup>b</sup>	4.69±0.09 <sup>ab</sup>	4.81±0.03 <sup>a</sup>
Chloride (mg/ml)	0.991±0.114 <sup>a</sup>	0.722±0.063 <sup>ab</sup>	0.699±0.033 <sup>b</sup>
pH	6.72±0.03 ns	6.66±0.03	6.66±0.01
r (min)	25.67± 2.25 <sup>a</sup>	20.94±2.09 <sup>ab</sup>	20.21±0.64 <sup>b</sup>
k20 (min)	2.47±0.49 ns	2.40±0.44	2.20±0.13
a30 (mm)	39.49±4.58 ns	40.06±3.89	41.80±1.22
a2r (mm)	47.95±2.50 ns	52.28±2.22	50.34±0.67

<sup>a, b</sup>  $p \leq 0.05$ .

**Table 3.** Estimated mean values for yield, components and coagulation properties of buffalo milk according to the three PMN classes

PMN (%)	<25	25-50	>50
TSCC ( $\times 10^3/\text{ml}$ )	129 $\pm$ 170 <sup>ab</sup>	88 $\pm$ 104 <sup>b</sup>	443 $\pm$ 95 <sup>a</sup>
bacterial count (cfu $\times 10^3/\text{ml}$ )	267 $\pm$ 141 ns	407 $\pm$ 86	475 $\pm$ 79
milk yield (L/morning milking)	5.95 $\pm$ 0.42 ns	5.40 $\pm$ 0.27	4.98 $\pm$ 0.24
Fat (%)	7.34 $\pm$ 0.35 ns	7.58 $\pm$ 0.22	7.72 $\pm$ 0.20
Protein (%)	4.51 $\pm$ 0.10 ns	4.55 $\pm$ 0.06	4.43 $\pm$ 0.06
Casein (%)	3.58 $\pm$ 0.10 ns	3.73 $\pm$ 0.06	3.62 $\pm$ 0.05
Lactose (%)	4.77 $\pm$ 0.07 <sup>ab</sup>	4.89 $\pm$ 0.04 <sup>a</sup>	4.72 $\pm$ 0.04 <sup>b</sup>
Chloride (mg/ml)	0.826 $\pm$ 0.091 <sup>ab</sup>	0.674 $\pm$ 0.066 <sup>b</sup>	0.878 $\pm$ 0.065 <sup>a</sup>
pH	6.62 $\pm$ 0.02b	6.65 $\pm$ 0.01 <sup>ab</sup>	6.69 $\pm$ 0.01 <sup>a</sup>
r (min)	18.49 $\pm$ 1.47 ns	19.67 $\pm$ 0.93	21.72 $\pm$ 0.85
k20 (min)	1.96 $\pm$ 0.31 ns	2.06 $\pm$ 0.20	2.43 $\pm$ 0.18
a30 (mm)	43.74 $\pm$ 2.68 ns	45.58 $\pm$ 1.71	39.89 $\pm$ 1.60
a2r (mm)	51.19 $\pm$ 1.47 ns	49.47 $\pm$ 0.94	50.14 $\pm$ 0.89

<sup>a, b</sup> p $\leq$ 0.05.

content was higher (p<0.05), and chloride content and clotting time were lower (p<0.05) than in positive samples. Notably, the bacterial count in samples that were negative for mastitis bacteria was lower (453 cfu $\times 10^3/\text{ml}$  vs. 647 and 660 cfu $\times 10^3/\text{ml}$ ), suggesting a positive relationship between milk hygiene and mammary health.

Table 3 shows that the PMN value was significantly related to the TSCC value, lactose and chloride content, and milk acidity (p<0.05). Table 4 shows the component values according to TSCC class. There was a significant increase in PMN values when the cell class changed from low (38%) to medium and high (56% and 64%)(p $\leq$ 0.05). Moreover, only 1% of the samples in the lowest TSCC class were positive for bacteria.

The correlation between TSCC and PMN (Table 5) was stronger (0.70) than that (0.32) found by Thomas et al. (2004); this correlation increased slightly (0.75) when only samples positive for bacteria were considered. The correlation decreased if values obtained using the reference method were used for the analysis (0.60).

PMN in buffalo milk increased in parallel with an increase in total cells, as reported by others (Guarino et al., 1994; Dhakal, 2004; Thomas et al., 2004; Piccinini et al., 2006).

Milk yield was negatively related to TSCC (p<0.05), confirming a report by Singh and Ludri (2001) and Ceron-Munoz et al. (2002). Significant changes in lactose and chloride content were also observed with increasing TSCC

**Table 4.** Estimated mean values for yield, components and coagulation properties of buffalo milk according to the three TSCC classes

TSCC ( $\times 10^3/\text{ml}$ )	<100	100-200	>200
PMN (%)	38 $\pm$ 2 <sup>b</sup>	56 $\pm$ 4 <sup>a</sup>	64 $\pm$ 3 <sup>a</sup>
Lymphocytes (%)	48 $\pm$ 2 <sup>a</sup>	31 $\pm$ 3 <sup>b</sup>	25 $\pm$ 3 <sup>b</sup>
Macrophages (%)	14 $\pm$ 1 ns	13 $\pm$ 3	11 $\pm$ 2
Bacterial count (cfu $\times 10^3/\text{ml}$ )	435 $\pm$ 70 <sup>ab</sup>	204 $\pm$ 148 <sup>b</sup>	666 $\pm$ 108 <sup>a</sup>
Milk yield (L/morning milking)	5.79 $\pm$ 0.21 <sup>a</sup>	5.14 $\pm$ 0.45 <sup>ab</sup>	4.73 $\pm$ 0.33 <sup>b</sup>
Fat (%)	7.35 $\pm$ 0.15 ns	7.23 $\pm$ 0.31	7.55 $\pm$ 0.23
Protein (%)	4.52 $\pm$ 0.05 ns	4.47 $\pm$ 0.10	4.38 $\pm$ 0.08
Casein (%)	3.65 $\pm$ 0.06 ns	6.63 $\pm$ 0.12	6.53 $\pm$ 0.09
Lactose (%)	4.87 $\pm$ 0.03 <sup>a</sup>	4.80 $\pm$ 0.07 <sup>b</sup>	4.64 $\pm$ 0.05 <sup>b</sup>
Chloride (mg/ml)	0.650 $\pm$ 0.039 <sup>b</sup>	0.862 $\pm$ 0.0862 <sup>ab</sup>	0.882 $\pm$ 0.068 <sup>a</sup>
pH	6.66 $\pm$ 0.01 ns	6.67 $\pm$ 0.02	6.69 $\pm$ 0.02
r (min)	19.82 $\pm$ 0.75 ns	20.75 $\pm$ 1.50	22.39 $\pm$ 1.16
k20 (min)	2.05 $\pm$ 0.16 <sup>b</sup>	2.21 $\pm$ 0.31 <sup>ab</sup>	2.72 $\pm$ 0.24 <sup>a</sup>
a30 (mm)	43.57 $\pm$ 1.32 ns	40.67 $\pm$ 2.67	32.23 $\pm$ 2.06
a2r (mm)	50.40 $\pm$ 0.77 ns	50.13 $\pm$ 1.56	48.42 $\pm$ 1.21

<sup>a, b</sup> p $\leq$ 0.05.

**Table 5.** Correlation between PMN and TSCC

	log10 TSCC (alternative method)	log10 TSCC (reference method)	
PMN (%)	0.70	0.60	All samples
	0.70	0.58	Negative for bacteria
	0.75	0.70	Positive for udder-specific and environmental bacteria

p<0.0001.

values (p<0.05). Both components are affected by mammary gland health, with lactose decreasing and chloride increasing in mastitis milk (Kitchen, 1981; Harmon, 1994; Pyorala, 2003). Some researchers have suggested that lactose levels are one of the best markers of mammary inflammation, proposing a threshold value of 4.7% (Hamann and Kromker, 1997; Hamann, 2002b); however, more practical experience is needed to determine the usefulness of this value (Pyorala, 2003).

Higher TSCC was associated with impaired rennet coagulation properties: The clotting time increased, and curd firming time (p<0.05) and firmness decreased. The same trend has been observed previously in studies of both cow (Grandison and Ford, 1986; Politis and Ng-Kwai-Hang, 1988c) and buffalo milk (Tripaldi et al., 2003).

## DISCUSSION

The analysis of buffalo milk in this study showed that when the TSCC was less than  $100 \times 10^3$ /ml, PMN comprised less than 50% of the total cells and the prevalence of mastitis from udder-specific bacteria was 1%. If the TSCC exceeded  $200 \times 10^3$ /ml, then PMN comprised over 50% of the total cells and the prevalence of mastitis was about 30%. Thus, as in previous studies (Dhakal, 2004; Piccinini et al., 2006), we confirmed that the threshold value of  $200 \times 10^3$  cells/ml in buffalo milk was indicative of the presence of inflammation. In addition, we found that the buffalo milk with less than  $100 \times 10^3$  cells/ml could almost always be considered "healthy."

These results are similar to findings for cow milk, where the threshold values have been defined for many years. Recent studies (Hamann, 2002a; Smith, 2002) have proposed that subclinical mastitis is indicated when the TSCC is over  $200 \times 10^3$  cells/ml and is not indicated when the TSCC is under  $100 \times 10^3$  cells/ml. Samples with TSCC values between  $100 \times 10^3$ /ml and  $200 \times 10^3$ /ml cells are more difficult to classify.

The DSCC results showed that when PMN cells was higher than 50%, total cells were much higher than  $200 \times 10^3$  cells/ml and the mastitis prevalence exceeded 60%.

There has been some doubt about the values of classes with PMN less than 50%, where the middle class had lower TSCC and chloride content and higher lactose content. It could be hypothesized that in buffalo milk in which PMN make up less than 50% of the TSCC, this type of cells did not correlate completely with SCC and the other milk components such as lactose and chloride. Both lactose and chloride varied when mammary inflammation was present, but they could be affected by factors other than inflammation that have not yet been identified (Kelly et al., 2000). These results confirmed those reported by Piccinini et al. (2006), who suggested that 50% PMN should be used as a threshold value for identifying subclinical mastitis.

Very low PMN values have been found in studies of buffalo milk from animals with healthy mammary tissue; these researchers proposed threshold PMN values that were less than 50% (Guarino et al., 1994; Thomas et al., 2004). The values observed in those studies were similar to those observed for cow milk, in which relatively few cells in milk from healthy mammary glands are PMN (12%) (Burvenich et al., 1995; Zecconi and Smith, 2003) but which increase when bacteria are present (Miller et al., 1991). In contrast, Silva and Silva (1994) observed that PMN were numerous even in buffalo milk from healthy animals. The discordant results regarding PMN counts in buffalo milk are probably due to the following: the great variability in PMN values, as discussed above; the methods used to differentiate mastitic and non-mastitic animals; the sample origin (quarter samples or total quarter sample); and the sampling method.

The clotting properties of milk from animals affected by subclinical mastitis varied more than characteristics that directly affected the clotting properties, such as pH and casein content (Storry and Ford, 1982; Ng-Kwai-Hang, 1986; Remeuf et al., 1991). We hypothesize that in addition to exerting an effect due to the resulting change in milk composition, an increase in TSCC has a direct effect on coagulation properties via increased enzyme activity (Politis and Ng-Kwai-Hang, 1988c).

## CONCLUSION

This study showed that in buffalo as in dairy cows, TSCC is a valid indicator of udder inflammation. Our results confirmed that a value of  $200 \times 10^3$  cells/ml should be used as the threshold value for early identification of an animal affected by subclinical mastitis. In addition to its association with significantly decreased milk yield, a TSCC value above this threshold value was associated with changes in milk composition and coagulating properties.

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