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Relationship between cellular and whey components in buffalo milk

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High somatic cell count (SCC) affects milk quality and cheesemaking, resulting in a reduction in cheese yield and quality. In dairy cows, quarter milk samples with >200 000 cells/ml are considered to have subclinical mastitis, while there is much uncertainty on the corresponding levels of SCC in buffalo milk. In this study 30 lactating water buffaloes were selected and SCC, differential somatic cell counts and several whey components were tested in quarter milk samples to assess the relationship between inflammation markers and milk quality. Overall 236 quarter milk samples were considered. To evaluate the relationship between cellular markers (SCC, polymorphonuclear leucocytes, PMN, and N-Acetyl-β-glucosaminidase, NAGase) and other milk components, three classes were defined (low, medium and high). Analysis of milk yield showed a significant reduction in the high class of each of the three markers chosen. Overall, the highest class was characterized by significant changes in milk composition and a lower milk quality. The presence of an inflammatory status of the udder was frequent after the first trimester of lactation and in buffaloes with two or more parturitions. This study showed that significant changes in milk components can be observed when SCC are >400 000 cells/ml, PMN are >50% and NAGase is >100 units. These thresholds could be suggested as levels to define udder health status in buffalo cows.

Keywords: Inflammation markers, SCC, NAGase, PMN.

Somatic cell counts (SCC) are recognized worldwide as a measure of udder inflammation (Smith, 2002). Indeed, the increase in leucocytes in milk and in the mammary gland, as a response to the invading pathogens or to their metabolites or toxins, leads to an increase in SCC. Therefore, SCC are used to assess udder health status, milk suitability for human consumption and to define premium/penalties on milk price (Reichmuth, 1975; Harmon, 1994). Moreover, high SCC affects the quality and shelf-life of pasteurized milk (Ma et al. 2000), and impairs cheesemaking through an increase of coagulation time and moisture content, and a decrease of curd firmness, resulting in a reduction in cheese yield (Politis & Ng-Kwai-Hang, 1988a, b, c; Cooney et al. 2000).

Based on the numerous data available for dairy cows, a quarter producing milk with >200 000 cells/ml is defined as subclinically mastitic, while bacteriologically negative quarters with <100 000 cells/ml are considered healthy (Smith, 2002; Pyorala, 2003). The scarceness of data for buffalo milk SCC in comparison with the dairy cow, leads to uncertainty about the level of SCC in buffalo milk that can be used to define the presence of an inflammation (Dhakal et al. 1992; Mahendra & Ludri, 2001; Ceron-Munoz et al. 2002; Pasquini et al. 2003). To identify the critical level, we chose to assess changes in milk components as proposed for cow milk (Hamann, 2002). This paper reports the results of a field study aimed at identifying suitable markers to define the inflammatory status of buffalo milk at quarter level.

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Materials and Methods

Animals and sampling

The study was performed at the Istituto Sperimentale per la Zootecnia in Rome (IZS) on 30 lactating water buffaloes

in spring and autumn 2003, selected from the IZS herd of 70 animals. The buffaloes were in a free stall barn and they were milked in a 12+12 herringbone parlour. Individual average daily milk production was 9·37 kg and the herd average SCC was $5{\cdot}08 \times 10^5$. Previous investigation showed that at least 35% of the buffaloes had *Staphylococcus aureus* intramammary infections.

Milk (100 ml) was taken from each quarter twice (one week apart), following standard procedures (NMC, 1999). At the laboratory of the ISZ, located close to the herd, the samples were spiked in different aliquots, as required by each analytical procedure.

Whey preparation

Whey was obtained from skimmed milk by centrifugation at 60 000 ***g*** at 4 °C for 30 min, and then it was aliquoted in 1500-µl tubes and immediately frozen at −80 °C for N-Acetyl-β-glucosaminidase (NAGase) and whey protein analyses. These assays were performed in a single session when all the planned samples were collected.

Total and differential cell counts

Somatic cells were counted on a Bentley Somacount 150 (Bentley USA). Differential somatic cell count (DSCC) was performed as follows. Milk (10 ml) was centrifuged at 1500 ***g*** at room temperature for 15 min, and then the tubes were left in a refrigerator for 20 min and the fat removed with a spatula. Whey and the remaining fat debris were removed by aspiration, and 50 µl of the sediment on the bottom of the tube was used to prepare the slides which were stained by the May-Grunwald-Giemsa method. Cells were differentiated by morphological characteristics as described by Yam et al. (1971) and Schalm et al. (1975).

Biochemical assays

NAGase was assessed in duplicate on whey by the procedure described by Kitchen et al. (1978) and expressed as units (pmol of 4-methylubelliferon released per min at 25 °C catalysed by 1 µl of milk) on a microplate fluorometer at 355 ex and 460 em (Ascent, ThemoLabsystem, Finland).

Serum protein electrophoresis

Whey proteins were assessed by agarose gel electrophoresis with Hydragel 15 HR (Sebia, France), which allows a higher resolution of electrophoresis in comparison with similar kits applied to serum proteins. This analytical tool is intended for separation of serum and whey proteins on automated multiparametric agarose gel electrophoresis system (Hydrasis, Sebia, France). The gels were analysed by a densitometer and dedicated software (Phoresis, Sebia, France). Protein standards were added as a reference for the densitometer analysis.

Table 1. Minimum, mean and maximum values for the three levels of N-acetyl-β-glucosaminidase (NAGase) and polymorphonuclear leucocytes (PMN) applied to classify milk quality

Component	Units		Minimum	Mean	Maximum
SCC†	cells × 10^{-3}/ml	Low	1	59	198
		Medium	212	288	391
		High	417	1702	8942
NAGase	units	Low	0·770	32·521	49·475
		Medium	50·318	70·201	99·766
		High	105·469	504·340	7624·900
PMN	%	Low	0·00	10·597	23·00
		Medium	26·00	41·023	50·00
		High	51·00	71·430	100·00

† Somatic cell count

Statistical analysis

Measurements were collected in a database and to evaluate the relationship between SCC and other milk components, three classes were defined (low: ⩽200 000 cells/ml; medium: 200 000–400 000 cells/ml; high: >400 000 cells/ml). The same approach was applied to NAGase and polymorphonuclear leucocytes (PMN), classifying them into three levels (low, medium and high) corresponding to the values reported in Table 1. Results were analysed by General linear model for repeated measures on SAS 8.2 (SAS/STAT, 2000) The between-subjects factor was represented by parity (3 levels), days in milk (3 levels), the interaction of these two factors, each of the three milk markers (PMN, SCC, NAGase; 3 levels), and the within-subjects factor was represented by samples for each quarter (2 levels). Comparison of means was performed by Tukey-Kramer test.

Results

A total of 236 quarter milk samples from 30 animals were considered. Table 2 reports the distribution of mean values for the different milk components, which showed large variations in the ranges for the different components.

DSCC analysis did not confirm the high level of epithelial cells reported by Dhakal et al. (1992), but the values observed for the different cell types were very similar to those reported for cow milk (Silva & Silva, 1994; Zecconi & Smith, 2003) and in other studies on buffalo milk (Silva & Silva, 1994). PMN were the most prevalent cells identified in milk with mean values of 47%, while lymphocytes (LYM) accounted for 33% and macrophages (MAC) for 18%. However, the range of values was very similar among the three cell types.

Milk yield was significantly reduced in the high class of each of the three markers chosen (Table 3). The distribution of quarters among the three classes of the selected markers by parturition and by days in milk (Table 4)

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Table 2. Mean values, SD and ranges for the different milk components in water buffalo quarter milk samples

Milk component	Units	Mean	SD	Range
SCC†	Log_{10}/ml	5·12	0·79	3·00–6·95
PMN‡	%	46·97	29·88	0·00–100·00
Lymphocytes	%	33·09	21·47	0·00–88·00
Macrophages	%	18·48	20·02	0·00–88·00
NAGase§	Log_{10}/ml	1·82	0·45	0·11–3·88
γ–globulins	%	4·60	2·48	0·30–14·00
Lactoferrin	%	3·49	1·81	0·40–11·39
Albumin	%	6·51	2·16	1·29–17·70
Lactalbumin	%	24·00	4·92	11·60–37·50
Lactoglobulin	%	6·51	2·16	1·29–17·70

† Somatic cell count
‡ Polymorphonuclear leucocytes
§ N-acetyl-β-glucosaminidase

Table 3. Least square mean values (±SEM) for milk yield respectively by the three classes of somatic cell counts (SCC), polymorphonuclear neutrophils (PMN) and N-acetyl-β-glucosaminidase (NAGase)

Milk components	*Level of milk component*		
	Low	Medium	High
SCC	$3·830 \pm 0·075^{a}$†	$3·140 \pm 0·180^{b}$	$2·936 \pm 0·110^{b}$
PMN	$3·810 \pm 0·116^{a}$	$3·558 \pm 0·137^{a}$	$3·138 \pm 0·096^{b}$
NAGase	$3·850 \pm 0·106^{a}$	$3·538 \pm 0·112^{b}$	$2·878 \pm 0·141^{c}$

† Values within a column with different superscript letters, are statistically different ($P<0·05$)

showed that the frequency of quarters in the low class for each parameter declined as parity and days in milk increased, while the frequency of quarters in the high class increased.

Relationship between SCC, NAGase, PMN and the other milk components

Table 5 reports the changes in milk components at the different levels of SCC considered. When SCC values were >400 000 cells/ml, significant changes in all milk components were observed. Indeed, significant increases in NAGase, albumin (ALB), γ-globulins (γG), lactoferrin (LF), and PMN and significant decreases in LYM, MAC, lacto-albumin (LA), lactoglobulin (LG) were observed.

Table 6 shows the values observed when NAGase classes were considered. Mean values of SCC, PMN and LA significantly increased as NAGase levels changed from low to medium and from medium to high. The opposite pattern, with a significant decrease, was observed for MAC and LA, while significant differences for ALB, γG and LF were found only in high NAGase class.

Analogously, Table 7 shows the mean values of the different milk components when PMN classes were considered. Significant increases for SCC and significant decreases for LYM and MAC were observed when PMN

Table 4. Frequency of quarters (%) in the three classes respectively of somatic cell counts (SCC), polymorphonuclear neutrophils (PMN) by parity and by lactation period

		Level of milk component		
Milk components	Parity	Low	Medium	High
SCC	1	41·7	12·5	22·1
	2	29·5	16·7	32·4
	3	28·8	70·8	45·6
PMN	1	55·6	25·6	27·1
	2	26·4	27·9	31·8
	3	18·1	46·5	41·1
NAGase†	1	45·3	34·7	12·1
	2	29·2	22·2	36·2
	3	25·5	43·1	51·7
		Days in lactation		
SCC	0–90	42·4	29·2	19·1
	91–150	28·8	58·3	29·4
	>150	28·8	12·5	51·5
PMN	0–90	48·6	23·3	29·9
	91–150	31·9	37·2	32·7
	>150	19·4	39·5	37·4
NAGase	0–90	63·2	18·1	0·00
	91–150	25·5	45·8	31·0
	>150	11·3	36·1	69·0

† N-acetyl-β-glucosaminidase

levels changed from low to medium and from medium to high. A significantly higher level of NAG, γG, and LF and a significant decrease in LA were observed in the high PMN class; in all the other cases no significant changes were observed.

Discussion

Previous research shows that after the first 90 d of lactation, the level of SCC increases progressively and that days in milk and parity have a significant influence on SCC (Ceron-Munoz et al. 2002). Our results confirm these findings with an increasing frequency of quarter in high SCC class (>400 000 cells/ml) as parturition and days in milked increased.

As expected, DSCC showed higher frequencies of quarters with >50% of milk PMN as lactation advanced. Even if part of this increase could be related to the normal activity of udder immune system (Burton & Erskine, 2003), the presence of large proportions of milk PMN in quarters from buffaloes in the second half of lactation and with more than two parturitions confirms the presence of an inflammation as reported in a similar investigation (Dhakal et al. 1992).

Distribution of quarters by NAGase classes was similar to that observed for SCC and for PMN. Indeed, an increasing number of quarters were in the high NAG class (>100 units) as lactation days and number of parturitions increased.

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Table 5. Least square mean values (±SEM) for the various milk components considered by the three classes of somatic cell counts (SCC)

		Level of SCC		
Milk components	Units	Low	Medium	High
PMN‡	%	36·984±2·381[a]†	58·909±5·549[b]	63·631±3·359[b]
Lymphocytes	%	38·502±1·854[a]	27·626±4·321[b]	26·731±2·616[b]
Macrophages	%	22·841±1·612[a]	11·339±3·758[b]	9·210±2·275[b]
NAGase§	Log_{10}	1·695±0·027[a]	1·903±0·064[b]	2·202±0·039[c]
γ–globulins	%	4·279±0·176[a]	4·010±0·419[a]	6·239±0·257[b]
Lactoferrin	%	2·986±0·130[a]	3·251±0·310[a]	4·723±0·190[b]
Albumin	%	6·140±0·182[a]	6·4677±0·433[a,b]	7·078±0·266[b]
Lactalbumin	%	24·667±0·283[a]	23·957±0·674[a,b]	22·827±0·413[b]
Lactoglobulin	%	58·204±0·454[a]	58·704±1·083[a]	54·688±0·665[b]

† Values within a column with different superscripts, are statistically different ($P<0·05$)
‡ Polymorphonuclear leucocytes
§ N-acetyl-β-glucosaminidase

Table 6. Least square mean values (±SEM) for the various milk components considered by the three classes of N-acetyl-β-glucosaminidase (NAGase)

		Level of NAGase		
Milk components	Units	Low	Medium	High
SCC‡	Log_{10}/ml	4·529±0·065[a]†	4·966±0·069[b]	5·971±0·087[c]
PMN§	%	34·361±3·182[a]	49·796±3·372[b]	64·515±4·557[c]
Lymphocytes	%	38·640±2·413[a]	32·755±2·558[a]	27·620±3·457[b]
Macrophages	%	24·283±2·085[a]	17·321±2·209[b]	7·458±2·986[c]
γ–globulins	%	4·121±0·226[a]	4·165±0·240[a]	6·636±0·302[b]
Lactoferrin	%	2·807±0·168[a]	3·129±0·178[a]	5·073±0·225[c]
Albumin	%	5·918±0·235[a]	6·611±0·249[b]	7·051±0·314[b]
Lactalbumin	%	25·242±0·364[a]	24·291±0·386[b]	22·162±0·487[c]
Lactoglobulin	%	58·314±0·599[a]	58·044±0·635[a]	54·452±0·800[b]

† Values within a column with different superscripts are statistically different ($P<0·05$)
‡ Somatic cell count
§ Polymorphonuclear leucocytes

Table 7. Least square mean values (±SEM) for the various milk components considered by the three classes of polymorphonuclear neutrophils (PMN)

		Level of PMN		
Milk components	Units	Low	Medium	High
SCC‡	Log_{10}/ml	4·558±0·082[a]†	5·075±0·097[b]	5·400±0·068[c]
Lymphocytes	%	50·705±1·913[a]	41·986±2·261[b]	19·056±1·584[c]
Macrophages	%	34·738±1·800[a]	14·928±2·127[b]	7·068±1·490[c]
NAGase§	Log_{10}/ml	1·726±0·047[a]	1·849±0·055[a]	1·948±0·039[b]
γ–globulins	%	4·412±0·279[a]	4·579±0·330[a,b]	5·227±0·231[b]
Lactoferrin	%	2·875±0·206[a]	3·457±0·243[a,b]	4·055±0·170[b]
Albumin	%	6·658±0·263[a]	6·501±0·310[a]	6·031±0·217[a]
Lactalbumin	%	25·191±0·686[a]	23·222±0·492[b]	23·730±0·345[b]
Lactoglobulin	%	57·191±0·686[a]	58·351±0·810[a]	56·788±0·568[a]

† Values within a column with different superscript letters are statistically different ($P<0·05$)
‡ Somatic cell count
§ N-acetyl-β-glucosaminidase

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In dairy cows, high levels of SCC, NAGase and PMN signify subclinical mastitis. Indeed, these milk components are well-recognized markers of inflammation (Pyorala, 2003). Our results suggest that the presence of an inflammatory status of the udder is frequent after the first trimester of lactation and in buffaloes with two or more parturitions. Distribution of SCC in dairy cows is quite variable in relation to the bacteria involved (Zecconi & Piccinini, 2002), and when the level of SCC is >200 000 cells/ml, the isolation of bacteria is uninfluential in defining the inflammatory status of the quarter, and significant changes in milk components can be observed (Hamann, 2002).

For quarters classified in the high class for SCC and NAGase, significant increases in PMN, γG and ALB were observed. The increase in ALB suggests a change in permeability in blood-milk barrier as a response to an inflammatory stimulus, and this change could affect milk composition and biochemistry (Ribadeau-Dumas, 1999). Moreover, the increase of LF suggests the presence of a progressive involution at epithelial level that could be related either to the presence of chronic inflammation (Sordillo et al. 1989a, b) or to premature starting of the drying-off period (Zecconi & Smith, 2003).

PMN were less accurate as a marker, owing to missing significant changes observed for ALB, LG and partially for NAGase, while the latter was confirmed to be the first marker of inflammation to change, and to be probably more sensitive than the other two markers. Indeed, significant changes were observed from low to medium class for SCC, PMN, MAC, ALB and LA. In regard to SCC, significant changes in milk components were observed only in the high class for γG, LF, LG and less for LYM, MAC and LA.

Overall, when the three different markers (SCC, PMN and NAGase) were considered, the highest class for each marker was characterized by significant changes in milk composition, and a lower milk quality. These changes suggest that cheesemaking and cheese yield could be affected, and investigations to assess these specific aspects are in progress.

From practical point of view, SCC represents the best compromise between accuracy and suitability. As already demonstrated in dairy cows, the threshold of 200 000 cells/ml could be used to early identify subclinical mastitis and the level of 400 000 cells/ml to define milk suitability.

As in dairy cows, also in buffaloes, significant changes in milk components can be observed when SCC are >400 000 cells/ml, PMN are >50% and NAGase is >100 units. These thresholds could be suggested as levels to define udder health status in buffalo cows.

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