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Effect of mastitis on raw milk compositional quality

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In this study, we investigated the impact of mastitis infection on the quality of milk composition in small-scale dairy bovine herds. The purpose of this study was to find a milk quality somatic cell count (SCC) standard that could be used as an integral component of a control program. In all, 396 quarter milk samples from lactating cross-bred cows (Holstein & Zebu) were analyzed; 56% of these quarters were experiencing intramammary infection, with an overall mean SCC of $5.46 \times 10^5 \pm 2.30 \times 10^4$cells/ml. Infected quarters had significantly ($p < 0.05$) higher mean SCC levels ($6.19 \times 10^5 \pm 4.40 \times 10^4$cells/ml) compared to healthy quarters ($2.65 \times 10^5 \pm 2.40 \times 10^4$cells/ml). In high SCC milk and infected quarters, the concentrations of non-casein fractions, sodium, chloride, and free fatty acid were higher ($p < 0.05$), while the casein content, lactose, casein-to-total protein, potassium, and calcium were lower ($p < 0.05$) compared to normal quarters. These findings suggest a mean SCC threshold limit of 5.46×10^5 cells/ml for the region. It was concluded that the results could be used to propose a milk quality SCC standard that can be used as an integral component of a control program.

Key words: compositional quality, raw milk, small scale farms, somatic cell count, subclinical mastitis

Introduction

The dairy cattle population in Kenya is estimated to exceed three million, and consists of indigenous breeds, i.e., *Bos indicus* and crosses between these species with exotic breeds, i.e., *Bos taurus* (e.g., Friesian, Ayrshire, Guernsey, and Jersey). The dairy industry is dominated by small-scale dairy farmers, who are estimated to contribute approximately 80% of total milk production [18]. Although increasing opportunities for income generation exist, small-scale dairy farms sometimes show sub-optimal animal performance, which could be attributed to poor management and disease [23].

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Mastitis, particularly the subclinical type, is one of the most persistent and widely spread disease conditions of importance to milk hygiene and quality among dairy cattle worldwide [5]. Mastitis influences the total milk output and modifies milk composition and technological usability. In cows, the somatic cell count (SCC) is a useful predictor of subclinical mastitis, and therefore, it is an important component of milk in terms of quality, hygiene, and mastitis control [7]. Elevated milk SCC is associated with altered protein quality, change in fatty acid composition, lactose, ion and mineral concentration, increased enzymatic activity, and a higher pH of raw milk [1,5].

Various studies have demonstrated that subclinical mastitis is a prevalent disease in smallholder dairy herds in Kenya [19,22] and elsewhere. This study evaluated the effect of subclinical mastitis infection on raw milk compositional quality in small-scale dairy herds. We sought to determine a milk quality SCC standard that could be used as an integral component of a control program within the region. The ultimate aim was to propose a milk quality improvement model as a management tool designed for use as part of a control program.

Materials and Methods

Study animal selection and sampling

A cross-sectional study was conducted between August 2004 and March 2006 in the Rift Valley province, a major milk production region of Kenya. In the study, farm selection was based on herd size, availability of crosses of Holstein Friesian and Zebu (*Bos indicus*) cattle, and willingness of farmers to participate. The grazing system was a cut-and-carry stall-feeding system where Napier grass and crop residues are the main feeds, with concentrate supplementation restricted to milking cows. The study herds were initially screened for mastitis using the California mastitis test, udder palpation, and visual examination of milk. From 311 cows of 54 herds meeting the breed selection criteria and available for sampling, 396 quarter milk samples from 99 lactating cows were obtained by stratified random sampling.

While the cows were restrained in a standing position,

quarter milk samples were collected after discarding the first few milliliters of milk. Udders and teats were cleaned with warm water and left to dry, and were then wiped with cotton buds soaked in 70% alcohol, after which a 5 ml milk sample for bacteriological culturing was aseptically collected in sterile tubes. Another aliquot was collected in 100 ml sample bottles and kept refrigerated at 4°C for SCC and chemical analysis. The Delvo test SP, a commercial screening test (GistBrocades, Netherlands), was used to screen the milk to ensure that no antibiotic residues were present in the milk. This test was performed as described by the manufacturer. It was necessary to confirm that the samples were free of antibiotics, for if antibiotics had already been prescribed and used prior to milking, some of the bacteria may have been inhibited. The samples were transported on ice to the Guildford Institute Laboratories of Egerton University, and were tested within 6 h of collection.

Bacteriological analysis

Bacteriological isolation and identification followed the procedures of the National Mastitis Council [16]. Using a sterile disposable culturing loop, 0.01 ml of milk was streaked onto half a plate of blood agar (Becton Dickinson, USA) supplemented with 5% ovine blood and incubated aerobically at 37°C. Plates were examined for bacterial growth at 24 and 48 h. Pure cultures were examined further for morphology, staining, and cultural characteristics, and for biochemical reactions. In cases where no growth was detected, plates were re-incubated at 37°C for an additional 24 h.

Bacteria were identified using standardized procedures [17]. In brief, catalase-positive cocci were identified according to colonial morphology, hemolysis production, Gram staining, growth in Baird Parker agar, fermentation of glucose, and mannitol and coagulase tube tests against a positive control of *Staphylococcus aureus* (*S. aureus*) subsp. *aureus* ATCC 12600 (Culture Collection of the University of Gothenburg, Sweden). Quarters were classified as non-infected (NI) if no organisms were isolated, and were determined to be infected if mastitis pathogens were isolated. Cultural identity results for other non-Staphylococci mastitis pathogens were not available in this study. Thus, non-identified pathogens were grouped in the other mastitis pathogen category.

Milk chemical analysis

For each of the quarter milk samples, all of the analytical assessments were carried out in duplicate; these assessments included pH (potentiometric method, Contort C830), SCC [10], lactose, and chloride [11]. The free fatty acid (FFA) content [9] was expressed as the acid degree value (ADV) (meqFFA/100 g fat). The total nitrogen (TN), non-protein nitrogen (NPN), and non-casein nitrogen (NCN) contents were determined using a Kjeldahl block digester apparatus (FossElectric, Denmark) and calculated as: TN = N × 6.38, NPN = N × 6.38, total protein (TP) = TN-NPN, NCN = N × 6.34 [19]. The Na and K content were measured by flame photometry, while the Ca content was measured by atomic absorption spectrophotometry [17].

Statistical analysis

SCC values were transformed to $\log_{10}$ prior to statistical analysis. Pearson correlation coefficients and linear regression were used to investigate the relationship between SCC levels and various milk components. The effects of SCC levels, infectious status, lactation stage, and lactation number on milk components were evaluated with ordinary least square means analysis of variance using the PROC GLM procedure in SAS statistical software [21].

Results

Bacteriological analyses

From the 396 quarter milk samples examined, 56% (n = 222) of the quarters were experiencing intramammary infection. Threshold limits of 3.50×10^5 SCC/ml have been fixed for milk quality control and udder health monitoring in the tropics [8]. Using this threshold limit, 76.27% of the quarters could be classified as infected.

The mean $\log_{10}$SCC in the individual milk samples was calculated based on locality, mastitic status, parity, stage of lactation, and previous history of mastitis. The study yielded an overall mean SCC of $5.46 \times 10^4 \pm 2.30 \times 10^4$ cells/ml and a standard deviation of 3.15×10^4, with 6.50×10^4 cells/ml and 2.20×10^6 cells/ml as the minimum and maximum SCC levels recorded, respectively. There were no significant differences ($p < 0.05$) between the mean SCC values for animals with different parities and at different stages of lactation. However, the locality significantly affected the mean $\log_{10}$SCC levels.

The SCC levels were significantly influenced by quarter health status. There was a significant rise ($p < 0.05$) in the mean SCC levels in the infected quarters, whereby healthy quarters had a mean $\log_{10}$SCC of 5.423 ± 0.02 ($2.65 \times 10^4 \pm 2.30 \times 10^4$) cells/ml compared to infected quarters, which had a mean $\log_{10}$SCC of 5.792 ± 0.03 ($6.19 \times 10^4 \pm 4.40 \times 10^4$) cells/ml. This was also reflected in quarters that had a previous history of mastitic infection exhibiting a significantly higher ($p < 0.05$) mean $\log_{10}$SCC than those with no previous mastitis history. In the infected quarters, the SCC responses also depended on the mastitic pathogen isolated. Quarters from which *S. aureus* was isolated had a higher ($p < 0.05$) mean $\log_{10}$SCC compared to other pathogens. The quarters from which coagulase negative *staphylococci*, and other mastitic pathogens were isolated had mean $\log_{10}$SCC values of 5.84 ± 0.03, 5.65 ± 0.04, and 5.79 ± 0.03 cells/ml, respectively.

SCC and milk composition

The qualitative parameters were analyzed according to the

Table 1. Mean variation of quarter milk components according to SCC threshold levels

Milk component	SCC thresholds (×1,000 cells/ml)			
	<250 A	250-500 B	500-750 C	>750 C
SCC, cell/ml	210,742[a]	374,030[b]	630,111[c]	1,025,781[d]
pH	6.63	6.70	6.69	6.81
ADV	0.23	0.29	0.45[a]	0.60[b]
TN, %	3.34	3.38	3.29	3.42
NPN, %	0.10	0.12	0.11	0.13
TP, %	3.32	3.12	3.18	3.25
NCN, %	0.64	0.65	0.70[a]	0.81[b]
CN, %	2.77[a]	2.59	2.59	2.57
CN/TP, %	83.6[a]	83.0[a]	81.2	78.9
Na, mg/100 g	46.5[a]	53.6[b]	66.2[c]	78.4[d]
K, mg/100 g	146.3[a]	139.5[ab]	128.6[b]	108.9[c]
Ca, mg/100 g	119.5[a]	114.0[b]	105.2[c]	97.8[d]
Lactose, g/l	48.8	47.9	45.1[a]	43.8[b]
Cl, mg/100 g	100.5[a]	116.3[b]	142.7[c]	183.5[c]

[a,b,c]($p < 0.05$).

SCC threshold classifications of the herd to test the effect of SCC on pH, FFA, milk protein fractions, and mineral content. The results relate to the mean values of the milk components at several SCC threshold levels are reported in Table 1.

The results show the SCC to have had no significant effect on the pH values of quarter milk samples, with the mean pH values falling within the normal milk pH range. However, the FFA content rose significantly ($p < 0.05$) along with the SCC level. The lower SCC threshold groups were not significantly different from each other, although milk with higher SCC levels showed a significant difference.

The acid degree value (ADV) was increased by 0.16-0.37 meq FFA per 100 g in higher SCC quarters (>5.0 × 10^5cell/ml). Furthermore, the FFA content had a high correlation (r = 0.75, $p < 0.05$) with SCC values in quarter milk (Fig. 1).

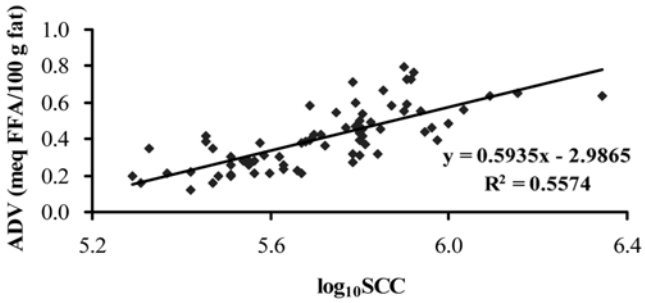

Fig. 1. The correlations between SCC and acid degree value (ADV) in quarter milk samples.

The protein fraction concentrations of the TN, TP, and NPN fractions were not significantly affected by SCC responses in the quarters. The Kjeldahl values for TP percentage according to SCC group were 3.32, 3.12, 3.18, and 3.25 for group A, B, C, and D, respectively. However, the percentage of the NCN fraction increased significantly ($p < 0.05$) with increasing SCC levels in quarters, while CN content was lower in SCC groups B, C, and D respectively. The elevation of the NCN fraction with a corresponding decrease in CN content resulted in an 1.8-4.5% decrease in CN/TP ratio in high SCC quarters (>5.0 × 10^5cell/ml). The CN/TP ratio (proteolysis index) was significantly affected ($p < 0.05$) by the SCC levels of quarters. Fig. 2 presents the correlations between SCC levels and TP, NCN, and CN/TP content ratios. Negative correlation was observed between the SCC level and the CN/TP ratio (r = –0.83) and NCN content (r = 0.70), respectively, at $p < 0.05$. The CN/TP (proteolysis index) was positively correlated with CN (r = 0.35, $p < 0.05$) and negatively correlated with NCN (r = –0.81, $p < 0.05$).

Mineral compositions (Na, K, Ca, and Cl) varied in the study herd, and were significantly affected by the SCC responses in quarters (Table 2). Pearson correlation coefficients calculated between $\log_{10}$SCC and various mineral components indicated a positive correlation with Na (r = 0.87) and Cl (r = 0.85), and a negative correlation with K (r = –0.64) and Ca (r = –0.87) at $p < 0.05$. Fig. 2 and Fig. 3 illustrate the correlation between SCC values and Na content, whereby approximately 75% variation in Na content in the quarter samples could be associated with the change in SCC content. Na content was positively correlated with Cl content (r = 0.77, $p < 0.05$), but negatively correlated with K content (r = –0.61, $p < 0.05$). The SCC and total protein components were positively correlated, though not as markedly as SCC and NCN content.

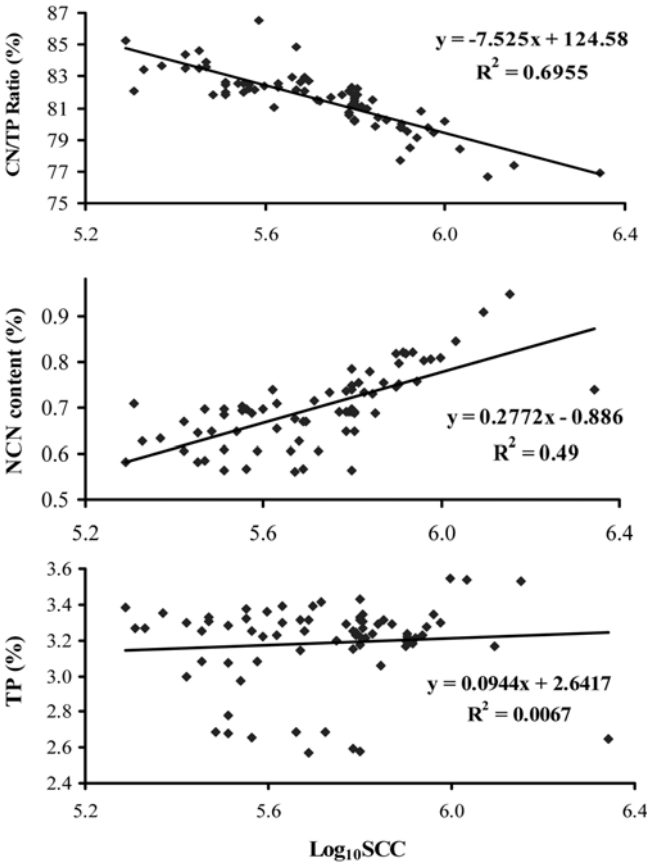

Fig. 2. The correlations between SCC and selected protein component contents in the quarter milk samples.

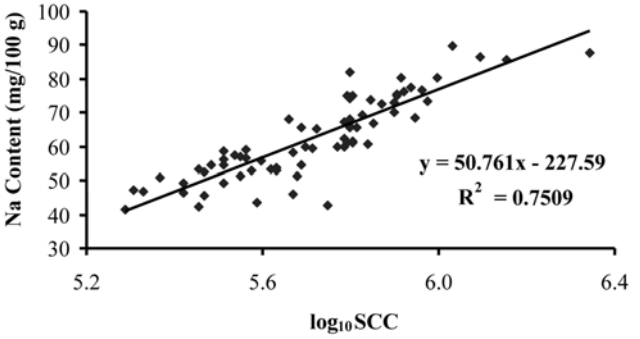

Fig. 3. The correlation between SCC and Na content in quarter milk samples.

The results related to the quarter milk physicochemical characteristics as affected by subclinical mastitis infection status, lactation stage, and parity are reported in Table 2. The pH and ADV values were higher ($p < 0.05$) in infected quarters than uninfected quarters. The mean ADV value of infected quarters was 1.3 times higher than healthy quarters, which reflected higher lipolytic activity in the infected quarters. However, the concentrations of TN, TP, and NPN fractions (Table 2) did not differ significantly ($p < 0.05$) between uninfected and infected quarters. Na content was significantly higher ($p < 0.05$) in infected quarters, with both minor pathogens (MIP) and major pathogens (MAP), compared to NI quarters (62.9 ± 3.46, 67.6 ± 3.08, and 54.2 ± 2.88 mg/100 g, respectively). However, no significant difference was observed between quarters infected with MIP and MAP. Mean CN/TP ratio, K content, and Ca content were lower in uninfected quarters than in infected ones ($p < 0.05$). Healthy quarters showed 1.19 and 2.17% higher CN/TP ratios than MIP- and MAP-infected quarters, respectively. The lactation stage did not significantly affect any of these parameters, whereas the parity effect was significant for SCC, Ca, and K.

Discussion

In this study, intramammary infections were present in 56% of the quarters examined, which indicated that subclinical mastitis is prevalent in smallholder dairy herds. This finding is comparable to those from studies of small scale herds in Kenya [19,22]. In these studies, the prevalence rate, which was based mainly on the mastitic pathogen, *S. aureus*, was determined to be between 30 to 45%. *S. aureus* subclinical mastitis is one of the most frequent and problematic types due to its chronic nature and its relative

Table 2. Raw milk compositional parameters according to quarter infection status, parity, and lactation stage effects

Quarter Status	Milk components										
	SCC	pH	ADV	TP	CN	CN/TP	Na	K	Ca	Lactose	Cl
Healthy	376,223	6.63	0.33	3.12	2.62	82.99	54.2	139.9	114.4	47.5	107.6
±SE	8,448	0.03	0.01	0.05	0.04	0.32	2.28	3.11	1.26	0.31	5.33
Infected	563,672	6.75	0.44	3.13	2.54	80.95	65.3	124.3	105.8	45.3	145.2
±SE	27,979	0.03	0.02	0.07	0.05	0.48	3.27	3.55	1.91	0.36	6.10
Effects											
Health status	<0.001	0.004	0.009	ns	0.044	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
Parity	0.048	ns	ns	ns	ns	ns	ns	0.01	<0.001	ns	0.002
Lactation stage	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns; non-significant ($p > 0.05$).

incurability in dairy herds.

The high SCC content in infected quarters as compared with uninfected quarters was similar to that reported by previous studies [3,4]. Due to the high prevalence rate of infection in small scale dairy herds, most quarters classified as uninfected are likely to have been subjected to several previous infections. This may explain the high background SCC content observed in this study. Somatic cells have been shown to maintain higher levels than before infection, even after the infection has been eliminated [5]. A mastitis diagnostic threshold limit of 6.50×10^5cells/ml has been suggested for the Kenyan dairy herds [18]. However, the findings from this study suggest that a threshold limit for acceptance based on mean SCC might be better placed at 5.40×10^5 cells/ml.

The mean value of milk components in the smallholder dairy herds irrespective of intramammary infection were found to be in agreement with published data from Europe and Africa [3,14]. However, most of the milk parameters showed strong mastitis-related changes depending on mastitis status. The contents of TN, TP, and NPN fractions in the present investigation were not significantly different between healthy and infected quarters. In contrast, the NCN fraction was found to be elevated, while the CN content was decreased, partly due to increased proteolysis leading to a decrease in the CN/TP ratio in infected quarters. This may linked to increased endogenous proteolysis due the elevation of plasmin or other proteases derived from somatic cells, leading to the breakdown of casein and the influx of blood proteins (immunoglobulins, IgG, and bovine serum albumin) into milk due to increased permeability of mammary epithelium, which results in an elevated NCN content [5,13].

High concentrations of Na and Cl, pH values and lower lactose, Ca, and K in high SCC and infected quarters found in this study were in agreement with the results of earlier studies [4,5]. In these two studies, the changes were thought to be linked to the reduced secretory activities of the mammary cells and increased permeability of the mammary epithelium. This can lead to the transfer of components from blood to milk, including citrates, bicarbonates, and Na and Cl ions. Higher levels of citrate and bicarbonate found during udder inflammation may be responsible for elevated pH levels [7].

In the present study, blood-borne electrolytes such as Na and Cl were higher in infected quarters, regardless of SCC levels, and seemed to pass into milk with a low level of udder disturbance. However, the lower Ca level found in infected quarters as compared with healthy quarters in the present study did not agree with other findings [5,12]. Furthermore, the Ca level was significantly decreased with increasing parity. As most milk Ca is associated with casein, reduced casein concentrations reported in the study could explain the lowered calcium levels in infected quarters. However, marked changes in Ca concentrations with no significant change in casein levels between quarters infected with minor and MAP in the present study were not found, and further study is warranted. Increased FFA during intramammary infections observed in the present study can be attributed to increased lipolytic activity due to increased lipase enzymes, and similar findings have been reported elsewhere [2,15]. Due to the high prevalence of intramammary infection in dairy herds, coupled with poor milk handling, processing procedures, and high temperatures, a higher FFA is expected in raw and pasteurized milk produced in Kenya, which may affect flavor quality and shelf-life.

In the case of the Kenyan dairy industry, where the bulk of milk produced is processed by pasteurization and ultra-heat treatment, changes in FFA, casein fractions, and mineral composition are of great importance. In other regions of the world, lowered raw and pasteurized milk quality with reduced shelf-life have been reported, while reduced shelf-life and organoleptic properties have been observed in ultra-heat treated milk from high SCC milk [1,15]. Although the use of SCC has increased as a means of milk quality control and udder health in industrialized countries, in Kenya and many countries in the tropics, this technique has not yet been adopted. As the high prevalence of subclinical mastitis in dairy herds presents a major constraint to high quality milk production, adoption of SCC for use in quality control is very important. Threshold limits of 3.50×10^5 SCC/ml have been fixed for milk quality control and udder health monitoring in the tropics [8].

The use of Pearson correlation coefficients and linear regression as used in this study made it possible to investigate the relationship between SCC levels and various milk components. The effect of SCC levels, infectious status, lactation stage, and lactation number on milk components was evaluated with ordinary least square means analysis of variance using the PROC GLM procedure in SAS statistical software [21]. Thus, the results provide new information on cross-bred (Holstein & Zebu) milk composition as affected by SCC levels, mastitis status, and parity. The presence of subclinical mastitis had a marked influence on milk compositional quality, and presents a major constraint to extensive high-quality milk production by small-scale dairy farmers. This study proposes a milk quality SCC standard of 5.46×10^5 cells/ml for the region; this standard can be used as an integral component of a control program. This is based on the mean SCC after the outliers were excluded and a rejection rate of 5% was given. The suggested threshold in this study is higher than that that used for the acceptance of bulk milk in Europe, New Zealand, and Australia, 4.00×10^5 cells/ml [6]. Considering the differences in breeds and climate production levels, a SCC standard of 5.46×10^5 cells/ml may be an a appropriate standard used for monitoring raw milk quality and udder health in small-scale dairy herds within the study region.

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