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Study on The Bulk Milk Somatic Cell Counts and Milk Quality in Different Seasons

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Driginal Research Article

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Abstract: The aim of this study were to investigate the effect of bulk milk somatic cell count on milk quality of Holstein-Friesian cows, to evaluate a seasonal factor that influences bulk milk somatic cell count and milk quality and to understand a relationship between bulk milk somatic cell count and milk quality. Milk Samples were collected from dairy farms at Pingtung, Taiwan during December 2016 to June 2017. All samples were analyzed for protein, fat, lactose, pH, titratable acidity, ionic calcium, ethanol stability, and total bacterial count (TBC). Milk samples from bulk tank in dairy farms were allotted into 3 groups according to total SCC, including group 1 with SCC below 200×10^3 /mL, group 2 with SCC ranged from 200×10^3 to 500×10^3 /mL, and group 3 with SCC ranged from 500×10^3 to 750×10³/mL respectively. The somatic cell count of bulk milk (BMSCC) influenced the milk quality in the dairy farm. The bulk milk with higher BMSCC showed higher fat, protein, ionic calcium content, pH, and TBC (p<0.01), and lower lactose content and ethanol stability (p<0.01). Total bulk milk somatic cell count between summer and winter season were significantly different (p < 0.05). Differences among seasonal milk fat, lactose, and ionic calcium contents were significant (p<0.05). The highest bulk milk somatic cell counts were observed in summer season. Therefore, bulk milk somatic cell count level in the dairy farm showed a good indication for evaluating milk quality, including fat, protein, lactose, pH, ionic calcium, ethanol stability and total bacterial count. Keywords: bulk milk somatic cell count, Holstein-Friesian cows, milk quality, milk composition

INTRODUCTION

Mastitis is one of the most common diseases in dairy cows and may result in great economic losses in the dairy industry [2]. Mastitis is defined as a mammary gland inflammation that is generally caused by bacterial infections. Mastitis remains as one of the top diseases that affects milk production. While cows with clinical mastitis can be easily identified by visible changes in milk composition and physical examination of the udder. Detection of subclinical mastitis is based on bacteriological examination of milk and assessment of udder inflammation. For the latter, one possible indicator is somatic cell count (SCC), which reflects the disease-combatting response of the animal to the pathogen [13].

Somatic cell count is one of the most important factors in evaluating milk quality and udder health. When there is bacterial infection or udder inflammation, the number of somatic cell count (SCC) in milk increases [21]. Increasing SCC in milk reduces the quality of raw milk and dairy products. For example, high SCC is also related to protein quality, change in fatty acid composition, lactose, ion and mineral concentration, increased enzymatic activity and a higher pH of raw milk.

SCC in milk is influenced by season, parity, age, stage of lactation, stress, milking interval, and environmental and managerial factors. The breed of cow also affects SCC in milk. However, the main factor affecting SCC is mammary gland infection [14]. Evaluating milk quality of dairy farm is based on bacteriological examination of milk and assessment of udder inflammation. For the latter, one possible indicator is somatic cell count (SCC), which reflects the disease-combatting response of the animal to the pathogen [13]. The bulk milk somatic cell count (BMSCC) is an indicator of the milk quality of dairy farm supplied to the company. Therefore, the aim of this study were to investigate the effect of bulk milk somatic cell count on milk quality of Holstein-Friesian cows, to evaluate a seasonal factor that influences bulk milk somatic cell count, and to understand a relationship between bulk milk somatic cell count and milk quality.

MATERIAL AND METHODS

Sample Collection

Ninety samples of bulk tank milk were selected from five dairy farmers in the region of Pingtung (Taiwan) during December 2016 to June 2017. Milk samples were collected into sterile bottles and were immediately carried to the laboratory. During milk collection and milk transfer to the laboratory, the milk samples were stored in the ice box to maintain milk quality and avoid microbial contamination.

Somatic Cell Count and Milk Composition

SCC in milk was measured using a Nucleo counter (SCC-100, Denmark). The milk compositions (fat, protein, and lactose) were analyzed by using Milkoscope (Scope Electric [®], Expert-2059, Germany).

Titratable Acidity and pH measurement

Titratable acidity was measured by titrating 8.8 mL of sample and 9 mL of distilled water with 0.1 N sodium hydroxide (NaOH) using 2 drops phenolphthalein solution in 95% ethanol as the indicator. The pH of milk samples was measured at room temperature using a digital pH meter (Suntex pH/ION meter SP-2500) after calibration of the pH probe.

Ethanol stability

Ethanol stability was measured by mixing equal volumes (1 mL) of milk sample and ethanol solution (water/ethanol ranging from 10 to 98%, v/v).

Ionic Calcium Concentration

Ionic calcium was measured using the Suntex pH/ION SP-2500 analyser at the room temperature.

Statistics

Statistical Analysis System (version 9.3, SAS) was used to perform data analysis using the general linear model (GLM). Duncan multiple range test was used for comparison of mean of treatments. Correlation coefficient was also established to investigate relationships between parameters.

RESULTS

Effect of somatic cell count on milk composition of bulk milk

A significant (P < 0.05) different between the three categories levels of SCC was found (Table 1). As expected, the level of BMSCC from dairy farmers showed great variations over sampling days. Mean BMSCC per herd during the study ranged from 192×10^3 to 662×10^3 cells/mL. Table 1 shows that the SCC in milk significantly influenced the fat, protein and lactose percentage (P < 0.001). Increase SCC were associated with increased fat and protein percentage, but lactose percentage decreased.

	Grouping of BMSCC (x10 ³ cells/mL)				
	Group A	Group B	Group C	SEM	
	<200	200-500	500-750		
n	29	26	26		
SCC (cells/mL)	192.350	366.816	662.933		
Fat (%)	3.54 ^a	3.69 ^b	3.79°	0.17	
Protein (%)	3.12 ^a	3.18 ^b	3.27 °	0.07	
Lactose (%)	4.71 ^a	4.60 ^b	4.51 ^c	0.13	

Table-1: Milk composition with different level of BMSCC

SEM : Standard error of mean.

^{a-c}Means within a row with different superscripts are significantly different (P < 0.05).

Effect of somatic cell count on Physicochemical and microbial properties of bulk milk

Table 2 shows that the SCC in milk significantly influenced the pH value (P < 0.001). The SCC in milk were not significant with the value of

acidity (P > 0.05). The bulk milk with higher BMSCC showed higher pH. Table 2 shows that the SCC in milk significantly influenced the ionic calcium and ethanol stability value (P < 0.001).

·	Grouping of			
	Group A	Group B	Group C	SEM
	<200	200-500	500-750	
n	29	26	26	
SCC (cells/mL)	192.350	366.816	662.933	
pH	6,64 ^a	6,68 ^b	6,75 [°]	0.02
$TA^{1}(\%)$	0,16	0,16	0,16	0.001
iCa^{2} (mM)	2.15 ^a	2.60^{b}	3.04 ^c	0.28
$\mathrm{ES}^{3}(\%)$	85 ^a	82 ^b	78°	1.88
TBC ⁴ (CFU/mL)	$7.2 \text{ x} 10^{3 \text{ a}}$	9.5 x10 ^{3 b}	$1.9 \times 10^{4 c}$	

Table-2: Physicochemical and microbial properties with different level of BMSCC

SEM : Standard error of mean

¹TA : titratable acidity. ²iCa: ionic calcium concentration. ³ES: ethanol stability. ⁴TBC: total bacterial counts ^{a-c}Means within a row with different superscripts are significantly different.

A significant (p < 0.05) difference between total bacterial counts of three somatic cell count categories of bulk milk was found (Table 2). Means of total bacterial count in SCC groups 1, 2 and 3 were 7.2 x10³ CFU/mL, 9.5 x10³ CFU/mL and 1.9x10⁴ CFU/mL, respectively. The high level of total bacterial count is an indication of high somatic cell count because poor hygienic conditions during milking.

Bulk milk somatic cell count and milk composition changes in different season

Comparing the means of somatic cell count showed significant differences in somatic cell count between winter and summer seasons (p < 0.05). Bulk milk somatic cell count in winter showed the lowest. BMSCC in winter, warm, and summer season were 380,150, 410,316, and 431,633 cells/mL, respectively.

Table-3: Means of milk fat, protein and lactose (%)	by season
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	Group of season			SEM
	Winter Warm Summer			
BMSCC (cells/mL)	380,15 ^a	410,316 ^{ab}	431,633 ^b	
Fat (%)	3.75 ^a	3.80 ^a	3.59 ^b	0.22
Protein(%)	3.17	3.20	3.19	0.10
Lactose(%)	4.77^{a}	4.58^{b}	4.49 ^c	0.12

SEM : Standard error of mean.

^{a-c}Means within a row with different superscripts are significantly different.

Means of milk fat and lactose contents in different seasons are given in Table 3. The differences of fat and lactose content between winter and summer season were significant (P < 0.05), but the protein content were not significant between winter and summer season.

content between winter and summer season was significant (Table 4). The seasonal effect was significant on total bacterial count. Means of total bacterial count in the winter, warm, and summer season were 8.5×10^3 , 1.1×10^4 , and 1.5×10^4 CFU/mL respectively.

Physical and chemical properties of bulk milk changes in different season

The seasonal effect was not significant on pH, titratable acidity, and ethanol stability; ionic calcium

Table-4: Means of p	oH, titratable acidity,	ionic calcium, ethanol stabilit	y and total bacterial counts by season
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	0	Group of season			
	Winter	Warm	Summer		
pН	6,69	6,66	6,69	0.05	
$TA^{1}(\%)$	0,16	0,16	0,16	0.001	
iCa ² (mM)	2.86 ^a	2.67 ^{ab}	2.41 ^b	0.59	
ES^3	82,4	83,4	82,4	3.56	
TBC^4 (CFU/mL)	8.5×10^{3} a	$1.1 \text{x} 10^{4 \text{ a}}$	$1.5 \times 10^{4 \text{ b}}$		
SEM : Standard error of mean.					

¹TA: titratable acidity. ²iCa: ionic calcium concentration. ³ES: ethanol stability. ⁴TBC: total bacterial counts ^{a-c}Means within a row with different superscripts are significantly different.

Correlation for relationship between BMSCC and milk composition

Correlation coefficients between somatic cell count level and milk compositions in bulk milk are listed in Table 5. Fat and protein content in bulk milk were significantly (p < 0.001) correlated with bulk milk

somatic cell count. BMSCC was signicantly correlated with lactose content (p < 0.01). BMSCC showed significant positive relationship to both fat and protein content in bulk milk. But a negative relationship to lactose content.

Table-5: Correlation coefficients (r) between bulk milk somatic cell count counts (TBMSC), and	l fat, protein and
lactose $(%)$	

	lactose (70)				
	Fat	Protein	Lactose		
BMSCC	0.491***	0.591***	-0.500***		
Fat	-	0.227 ns	-0.153 ns		
Protein		-	-0.353***		
Lactose			-		

P* < 0.05; *P* < 0.01; ****P* < 0.001. ns, not significant.

Correlation for relationship between SCC and physical and chemical properties of bulk milk

Correlation coefficients between somatic cell count and physico-chemical properties of bulk milk are listed in Table 6. pH and ionic calcium of milk were significantly positive (p < 0.01) correlated to bulk milk somatic cell count. BMSCC was signicantly negative correlated to ethanol stability (p < 0.001). BMSCC did not correlated to titratable acidity.

Table-6: Correlation coefficients (r) between b	oulk milk somatic cell count	counts (TBMSC), and pH, TA, iCa and
	$\mathbf{EC}(0/1)$	

	ES (%)			
	pН	TA	iCa	ES
BMSCC	0.870***	0.142 ns	0.749***	-0.833***
pН	-	-0.215*	0.388 **	0.687***
ТА		-	0.171 ns	-0.145 ns
iCa			-	-0.660***
ES				-

P* < 0.05; *P* < 0.01; ****P* < 0.001. ns, not significant.

DISCUSSION

Numerous studies, an high SCC is related to changes in the composition of milk. This change may have two main explanations [18], Including injury of udder cells which reduces the synthesis of milk constituents in the udder, and changes in permeability of membranes and interstitial spaces, that increase the passage of components from blood to milk.

The bulk milk with higher BMSCC showed higher fat. Rajcevic [15] indicated that the most important enzymes that somatic cells produce in response to mammary glands infection are lipolytic enzymes. Gargouri [8] showed a positive correlation between lipolytic enzyme activity and somatic cell count in milk. These lipolytic enzymes damage the milk fat globule membrane, exposing milk fat to degradation by lipoprotein lipase in the milk. This increase in lipolysis leads to elevated levels of free fatty acids, which can cause development of rancid flavours in the milk [13].

The bulk milk with higher BMSCC showed higher protein. The effects of SCC on protein constituents in cow milk are numerous and sometimes

conflicting. Rajcevic [15], Najafi [13], and Yarabbi [20] reported that cow milk with a high SCC contained more total protein than milk with low SCC. On the contrary, Sharma [18] found total protein content was the lowest in milk with the highest SCC levels. Botaro [5] found no significant differences between protein contents of milk with high or low SCC. Increases in the concentration of proteins from blood during mastitis lead to an increase in the concentration of soluble whey proteins [14]. Yarabbi [20] found a decrease in casein content when SCC increases, while Atakan [1] noted an increase in casein with an increase in SCC. High SCC in cow milk was also associated with higher soluble protein contents [17]. However, it is important to note that mastitis may cause a decrease in the casein content and/or an increase of serum protein levels in milk, depending on the severity of the process [7]. Therefore, the balance on casein and serum protein contents in milk could be responsible for confounding effects in each study.

The bulk milk with higher BMSCC showed lower lactose. Several studies have reported decreases in lactose concentration in the milk of cows presenting high SCC [6]. A negative correlation was observed between the percentage of lactose in milk and the severity of the disease [20]. According to Garcia [6], mastitis determines a continuous reduction in lactose concentration in milk with SCC above 100,000 cells/mL. when SCC increases from 83,000 cells/mL to 870,000cells/mL, lactose concentration is reduced from 4.977% to 4.707%.

The bulk milk with higher BMSCC showed higher pH. Ogola [14] reported that the changes of pH 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.

The bulk milk with higher BMSCC showed higher ionic calcium. It is interesting to note that ionic calcium show a wide variation in different bulk milk SCC. It has been previously stated Tsioulpas [18] that the average Ca^{2+} concentration in milk is about 2 mM. However, results from this study, showed that, the average free Ca^{2+} concentration was slightly higher, around 2.34 - 2.98 mM. Lewis [10] found that the mean value of free Ca^{2+} for sub-clinic mastitis was 2.90 mM, early lactation milk was 4.25 mM, and mid lactation milk was 2.90 mM. The lowest value found for bulk milk Lin [11] study was 1.96 mM.

The bulk milk with higher BMSCC showed higher ethanol stability. There was a significant negative relationship between ionic calcium and ethanol stability in milk. High ionic calcium causes the negative charge to reduce and promotes instability at lower concentration of ethanol [12]. Milk ethanol stability (MES) was defined as the minimum concentration of added aqueous ethanol that gives rise to milk coagulation [4]. Rathnayake [16] thoroughly discussed newly proposed theories connected to MES and clearly showed that mechanisms involved in it were complex and not totally elucidated. the necessity of transferring this knowledge to formulate new dairy products or to extend the shelf life of the existing products such as cream liqueurs or alcoholic beverages [5].

The effects of seasonal variation on milk composition have reported that the concentrations of many constituents and the physico-chemical properties vary throughout the year to different extents [13]. Rajcevic [15] reported that fat and lactose contents were loer in summer than in winter milk, which could be attributed to the different temperatures. The differences might be caused by changes in temperature among seasons in the region of the study. Fat and lactose contents were lower in summer season compared to winter season [9]. A negative correlation (p < 0.001) was observed between SCC and lactose, which is in accordance with previous results from Yarabbi [21] that SCC negatively affected lactose content. Reduction in lactose contents in milk presenting high SCC. According to Rajcevic [15]. Mastitis disease causes decreasing milk lactose through damaging the secretary cell that produce milk in mammary glands.

CONCLUSION

Somatic cell counts of bulk milk (BMSCC) influenced the milk quality in the dairy farm. The bulk milk with higher BMSCC showed higher fat, protein percentage, ionic calcium content, pH, and TBC (p <0.01), but lower lactose percentage and ethanol stability (p < 0.01). Significant difference was found between total bulk milk somatic cell count in summer and winter season. Differences among seasonal milk fat, lactose percentage and ionic calcium contents were significant (p < 0.05). The milk samples from infected quarter showed higher fat, protein percentage, pH, ionic calcium content and TBC (p < 0.05), but lower lactose percentage, titrable acidity and ethanol stability (p <0.05). Therefore, somatic cell count level in the dairy farm showed a good indication for evaluating fat, protein, lactose, pH, ionic calcium, ethanol stability and total bacterial count.

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