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Original Article

Relationships among malondialdehyde, milk compositions, and somatic cell count in milk from bulk tank

Witaya Suriyasathaporn[1,*], Usanee Vinitketkumnuen[2] and Teera Chewonarin[2]

[1] *Department of Food Animal Clinic, Faculty of Veterinary Medicine,*

[2] *Department of Biochemistry, Faculty of Medicine,*
Chiang Mai University, Muaeng, Chiang Mai, 50200 Thailand.

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Abstract

The goals of this study were to identify associations of malondialdehyde (MDA) with milk compositions and somatic cell counts (SCC) in milk from bulk tanks. Milk samples were collected from small-holder dairy farms (n = 133) belonging to the Mae-On dairy cooperative, Chiang Mai Province, Thailand. After routine testing for bulk tank SCC (BMSCC), milk samples were tested for milk compositions and milk MDA, respectively. To normalize the BMSCC data, they were transformed to scores of BMSCC. Results from Pearson's correlation coefficients showed that any pairs of BMSCC, milk fat, milk protein, and milk lactose were associated to each others (P<0.05). However, milk MDA was significantly associated only with BMSCC. In conclusion, milk malondialdehyde is associated only with somatic cell counts.

Keywords: bulk milk somatic cell count, malondialdehyde, milk fat, milk protein, milk lactose

1. Introduction

Milk has many nutritious qualities that make it an important part of children's diet. To produce the best quality milk and to achieve all the nutritious benefits of it, the highest quality raw milk must be obtained. Somatic cell count (SCC) has long been viewed as a key factor in the quality assessment of raw milk in the international dairy industry. When milk from a bulk tank has a high SCC value it is an indication that at least a portion of the milk was derived from mastitic cows. The EU, New Zealand, and Australia require that milk used for dairy products must have a bulk milk SCC (BMSCC) of <400,000 cells/ml, Canada <500,000 cells/ml, and the USA <750,000 cells/ml (for review see Schukken *et al.*, 2003).

An increased SCC value is correlated with an increased amount of the heat-stable protease (plasmin) and lipase (lipoprotein lipase) in milk (Azzara and Dimick, 1985, for review see Barbano *et al.*, 2006). Though starting with raw milk that has a low bacterial count without any microbial growth in pasteurized milk; if the milk has a high SCC it will lead to increased levels of protein and fat degradation during refrigerated storage, subsequently producing off-flavors (for review see Barbano *et al.*, 2006). Recently, it was demonstrated that mastitis adversely affects the quality of pasteurized fluid milk by accelerating the development of sensory defects such as rancidity and bitterness (Ma *et al.*, 2000). Off-flavors in dairy production are not only caused by an increased amount of plasmin and lipase in the milk, but the formation of lipid oxidation products also produce off-flavors and are correlated with the loss of nutrients.

In general, autoxidation represents a problem for all fat/oil-containing foods. The deterioration of lipid-rich foods is correlated with the initial formation of peroxides, the unstable and reactive primary products of lipid oxidation,

*Corresponding author.
Email address: suriyasathaporn@hotmail.com

and with their breakdown which leads to a combination of secondary products (mainly aldehydes and ketones). The most important secondary product of autoxidation is malondialdehyde (MDA), which is usually used as an indicator of the lipid peroxidation process. In milk, MDA concentrations were measured to evaluate the peroxidation levels when milk was kept under different conditions (Cesa, 2004; Miranda *et al.*, 2004). With regard to the quality of raw milk, Suriyasathaporn and colleagues (2006) have shown that milk MDA is associated with SCC in raw milk from cows. There is no study, however, on the association of milk MDA and SCC in a bulk milk tank. Therefore, the objective of this study was to identify the association of milk MDA and SCC at herd level.

2. Materials and Methods

Milk samples were collected in May 2006 from small-holder dairy farms (n = 133) in the Mae-On dairy cooperative, Chiang Mai Province, Thailand, during their regular milk quality control practices. In general, controls of milk quality have been performed by their cooperative using BMSCC and methylene blue dye reduction test, which determines the price at which the milk can be sold. Milk samples for BMSCC have been collected approximately once a week, and the day of sampling each week is chosen randomly. After routine testing for BMSCC using a Fossomatic cell counter (Foss Electric Ltd., Hillerod, Denmark), milk samples were first tested for milk composition, and were then immediately transported to the laboratory of the Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Thailand, for the measurement of MDA. Milk compositions, including milk lactose, protein and fat, were determined using a Milkoscan 133B (Foss Electric, Hillerød, DK-3400, Denmark).

Milk MDA was measured by the modified Smith's method (Santos *et al.*, 1980). Briefly, a hundred microliters of milk was properly mixed with 1 mL of 10% trichloroacetic acid (TCA) with a vortex mixer. Afterward, 400 µL of 2% wt/vol thiobarbituric acid (TBA) was added. The mixture was boiled for 30 min and subsequently cooled down by tap water. The solution was measured four times by UV spectrophotometry at 532 nm against its blank reaction mixture (without TBA). The average of optical densities obtained from each sample was used to define the MDA concentration.

To normalize the BMSCC data, they were converted to a BMSCC score by taking log2 of (SCC/100,000). To detect multicollinearity, Pearson correlation coefficients were calculated among variables including the BMSCC score, milk lactose, milk protein, milk fat, and milk MDA.

3. Results and Discussion

Medians, means, and standard error of means (SEM) of lactose, protein, fat, BMSCC, and MDA are shown in Table 1. Milk components reported in the current study were in the range as reported in Ballou *et al.* (1995). The average score of SCC was 4.40 that was higher than that of a study during the dry season in Thailand (Chawalkul and Suriyasathaporn, 2001), but it was in the range of BMSCC during 1985-1988, the period without the penalty program in Ontario (Schukken *et al.*, 1992). A seasonal effect has been reported for SCC contribution (Allore *et al.*, 1997; Suriyasathaporn *et al.*, 2002; Sewalem *et al.*, 2006). However, the effect of season on SCC is minor if the gland is uninfected, and the infection status has the most significant effect on milk SCC (Harmon, 1994). It is also possible that the BMSCC penalty program is quite weak in the Mae-On dairy cooperative. Most farms (62.3% of all farms) in this study had scores of BMSCC >4 (or BMSCC > 200,000 cells/ml). The high level of BMSCC in most farms might be attributed by the weak BMSCC penalty program. In the Mae-On dairy cooperative, the penalty is approximately 1% on the overall milk price when the BMSCC increases 200,000 cells/ml and the maximum penalty is limited to 4% of the overall milk price. The average MDA at herd level in this study was 1,572 ppb (Table 1). The level reported here is in the range at cow's level in our previous study (Suriyasathaporn *et al.*, 2006). The evaluation of accuracy for milk MDA data obtained from the TBA-test compared with an HPLC derivative method

Table 1. Median, mean, and standard error of means (SEM) of milk compositions including lactose, protein, fat, bulk milk somatic cell count (BMSCC), and milk malondialdehyde (MDA). Data from bulk milk samples collected from small-holder dairy farms in Mae-on dairy cooperative, Chiang Mai Province, Thailand (n = 138).

Variable	Mean	SEM	Minimum	Median	Maximum
Lactose (%)	4.85	0.012	4.45	4.86	5.15
Protein (%)	2.97	0.016	2.58	2.96	3.54
Fat (%)	3.83	0.036	2.81	3.77	5.99
BMSCC (cells/ml)	412	36.7	32	265	2,336
Score of SCC	4.40	0.12	1.36	4.41	7.55
MDA (ppb)	1,572	15.0	1207	1,577	2,065

Table 2. Multicollinearity among milk compositions including fat, protein, lactose, bulk milk somatic cell count (BMSCC), and milk malondialdehyde (MDA), resulting from Pearson's correlation coefficient.

	Fat	Lactose	Protein	MDA
Lactose	-0.1864*			
Protein	0.5363**	-0.0335		
MDA	0.0301	-0.0703	0.0972	
Score of BMSCC	0.2246**	-0.3396**	0.2772**	0.2187**

* P < 0.05, ** P < 0.01

showed a good agreement between the two analytical techniques (Cesa, 2004). The derivatization of MDA with the TBA reagent is a non-specific quantitation and lead to the overestimation of the MDA content (for a review see Janero, 1990). However, all samples in this study were processed in the same environment; therefore, any environmental and technical effect should therefore be identical. The relationships between MDA and milk quality found are thus not influenced by those effects

The relationships among score of BMSCC, milk components, and MDA are shown in Table 2. Most pairs between milk components were correlated to each other, excepted for MDA that was only correlated to the scores of BMSCC. With increasing BMSCC, fat percentage, protein percentage, and MDA increased ($p<0.01$), but the lactose percentage decreased. Several studies have shown a negative correlation between SCC and lactose in both quarter level (Lindmark-Månsson *et al.*, 2006) and farm level (Schukken *et al.*, 1992; Suriyasathaporn and Chawalkul, 2002). The content of fat increased significantly with elevated SCC measured in both quarter level (Sarikaya *et al.*, 2006) and farm level (Suriyasathaporn and Chawalkul, 2002). Lactose concentrations showed the opposite tendency to fat with increasing SCC (Bruckmaier *et al.*, 2004). In the central part of Thailand, BMSCC was also positively associated with milk protein (Suriyasathaporn and Chawalkul, 2002). In contrast, a decrease in BMSCC had little association with protein percentage (Mitchell *et al.*, 1986; Schukken *et al.*, 1992). However, a study in goats showed that casein concentration did not differ, whereas they protein and albumin concentrations were significantly higher in the infected glands than in the uninfected glands (Leitner *et al.*, 2004).

This paper is the first publication showing that MDA was only associated with BMSCC, but not others milk components. The association between MDA and BMSCC found in this study is in agreement with our previous study at cow level (Suriyasathaporn *et al.*, 2006). Somatic cell counts greater than 200x103 cells mL^{-1}, have been used as an indicator for udder-inflammation (Schepers *et al.*, 1997). In our data, the level of SCC in milk was positively associated with milk MDA. Milk with higher SCC has more infiltrated PMNs in the milk, and this causes an increase of oxidative reactions (Su *et al.*, 2002) and apoptosis (Tian *et al.*, 2005). It is as expected that no association between other milk components and MDA was found. In general, some sources of biosynthesis of milk components derive from blood circulation, especially triglycerides and glucose for synthesis of milk fat and lactose (Park and Jacobson, 1993). Reactive oxygen metabolites generated during normal metabolism and this metabolites stimulated by xenobiotics can enter into reactions that, when uncontrolled, can impair performance of dairy cows (for a review see Miller *et al.*, 1993). In this study, milk samples were originated from a bulk tank assuming that most of milk was derived from normal cows.

In conclusion, levels of MDA in milk are associated only with SCC at farm levels. As MDA is a stable product, MDA can be measured in finished dairy products such as formula milk (Cesa, 2004; Miranda *et al.*, 2004). The MDA levels found in the infant milk formulas ranged approximately between 200 and 1200 ppb or approximately 6 times in differences (Cesa, 2004). It is interesting to determine whether the differences of MDA levels in dairy products are caused by the differences in MDA levels of raw milk.

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