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The Effect of Different Somatic Cell Levels on Calcium and Phosphorus Contents of Milk

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ABSTRACT: Mastitis is an inflammatory reaction of the mammary tissue in response to the introduction and multiplication of pathogens and is characterized by increasing somatic cell count (SCC) in milk. The main objective of present study is to assess the probable associations between somatic cell (SC) levels and calcium and phosphorus and ratio of Ca/P in raw milk. Milk samples were collected from individual quarters of 30 Holstein cows and divided into three groups based on SC level, including: low SC level (<200,000), intermediate SC level (200,000 – 800,000) and high SC level (>800,000 cells ml^{-1}), followed by calcium and phosphorus analysis. Significant negative association between SC level and Ca, P, Ca/P ratio and titratable acidity (TA) of raw milk were found, while pH increased significantly by increasing SC level. It might be concluded that nutritional quality of milk and milk products strongly depends on the establishment of effective control measures for SC levels in raw milk.

Keywords: *Calcium, Mastitis, Phosphorous, Raw Milk, Somatic Cell Level.*

Introduction

Provision of calcium and phosphorus reflects one of the most important nutritional aspects of milk and milk products consumption worldwide. Nutritional values of dairy products may deteriorate due to incidence of animal disease mainly mastitis (Jones and Bailey, 1998).

Although symptoms of clinical mastitis are easily observable either in milking cow and fresh raw milk, probable abnormalities in milk from cows with sub-clinical mastitis should be monitored by laboratory tests such as SCC (Korwin-Kossakowska, 2006).

Mastitis especially sub-clinical one which is specified by increased SC level, causes a serious interruption in dairy industry, including inferior product quality, lower processability and decreased cheese making yield. Additionally the quality and quantity of milk proteins particularly casein as well as fat, lactose and minerals might alter by increased level of SC.

The followings are three main consequences of mastitis (Jones and Bailey, 1998):

- Increased permeability between blood and milk in secretory cells
- Decreased synthesis ability of mammary glands
- Higher enzymatic activity in milk

Due to lower synthesis of milk components in epithelial cells and especially decreased permeability of blood-milk barrier, the concentrations of Ca and P might change which could affect the nutritional quality of milk and milk products.

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Although previous researches have demonstrated the impact of clinical and sub-clinical mastitis on raw milk quality, several studies have been conducted on calcium content in clinically mastitic milk and no published data has been available regarding the probable association between calcium, phosphorus and Ca/P ratio, and SC level in sub-clinically mastitic milk. Therefore the main objective of this study was to determine the relationship between SC level, and Ca and P content and Ca/P ratio.

Materials and Methods

- Milk sampling

Milk samples were collected from the morning milking of a commercial dairy farm located in Tehran province. Milk sampling was done from every quarters based on ISO 707 and AOAC 968.12 (AOAC 2002 and ISO 1997). Therefore 30 pure Holstein lactating cows were selected which had the same age (4 years old), lactation period, number of calving and diet, without antibiotic injection seven days before sampling. The samples were transferred to Iran Animal Breeding Center laboratory located in Karaj rapidly at 4±1^{o}C to determine SCC by fossomatic cell counter (Fossomatic 150, USA). Therefore milking cows were classified in three groups: low (<200,000), intermediate (200,000–800,000) and high SC level (>800,000 cells ml^{-1}).

Test samples were collected in bottles which had been soaked in sulfochromic solution and rinsed with deionized water overnight. The samples were transferred to the laboratory at 4±1^{o}C immediately for chemical analysis.

- Calcium and phosphorus determination

One milliliter of each milk sample was poured in a small beaker and after the addition of 10 ml of nitric acid (65%), each beaker was covered with a watch glass and left overnight. The samples were heated on electric heater until a few milliliters of solution remained. The solutions were allowed to cool in room temperature and after the addition of 6 ml of hydrogen peroxide (30%), reheated until a few amounts of solution remained. Samples were diluted and calcium concentration was determined using Flame Atomic Absorption Spectrometry (FAAS) (Pohl and Prusisz, 2007; Rodri'guez and Sanz Alaejos, 2001).

Phosphorus concentrations in milk samples were determined according to ISIRI method 1260 (ISIRI, 2003).

- pH and titratable acidity measurement

pH and TA of the samples were determined according to AOAC methods 981.12 and 947.05, respectively (AOAC, 2002).

- Statistical analysis

All the data were subjected to one-way analysis of variance (ANOVA) using SAS and Minitab software. Duncan's new multiple range tests was used to separate significant treatment means.

Pearson correlation matrix showed the statistical relationship between all test data.

Results and Discussion

Mastitis incidence and increased SC level reduced calcium and phosphorus contents, ratio of Ca/P and TA, while positive association between pH and SC levels was found (Table 1).

-Milk calcium content

The amount of calcium in milk showed a reduction by increasing the SC levels (Figure 1) and a significant difference was observed in calcium content in all three SC levels (α=0.01). Also according to linear regression diagram (Diagram 1), approximately 81% of the changes in calcium content of milk samples can be attributed to increased SC levels (R^2 = 0.81).

Table 1. Experimental data in three levels of SC

Experimental objects	Low SCC (SCC < 200000)	Medium SCC (200000 < SCC < 800000)	High SCC (SCC > 800000)
Calcium Content (mg L^{-1})	$1107.70^{a}\pm89.93$	$908.55^{b}\pm88.35$	$715.2^{c}\pm64.69$
Phosphorus Content (mg L^{-1})	$820.63^{a}\pm30.64$	$729.43^{b}\pm47.21$	$633.16^{c}\pm22.87$
Ca/P Ratio	$1.38^{a}\pm0.06$	$1.24^{b}\pm0.05$	$1.13^{c}\pm0.07$
pH	$6.66^{a}\pm0.04$	$6.72^{a}\pm0.02$	$6.81^{b}\pm0.08$
TA (^{o}D)	$15.20^{a}\pm0.66$	$13.25^{a}\pm0.50$	$11.98^{a}\pm0.45$

Note: $^{a, b, c}$ Different for α=0.01

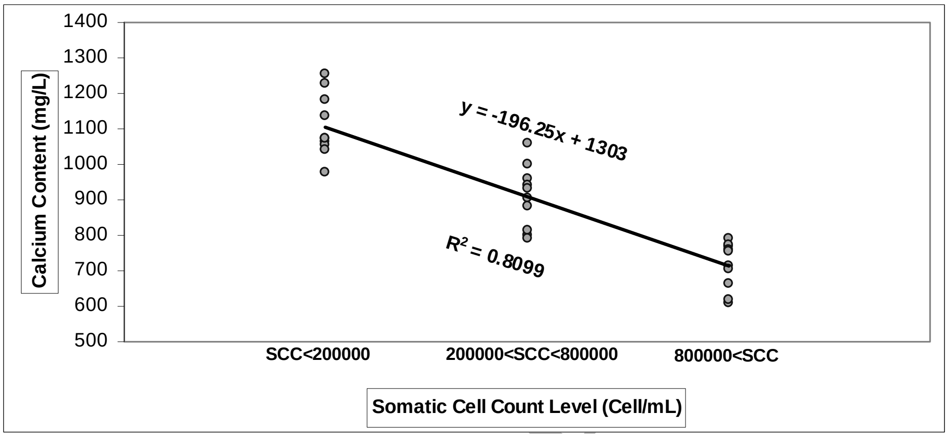

Diagram 1. The linear regression of SC level effect on calcium content of milk samples

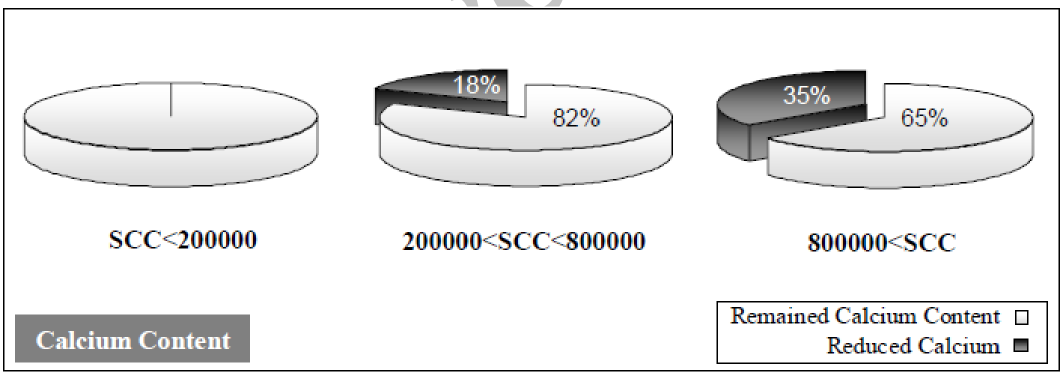

Fig. 1. The percentage of calcium content reduction in milk samples in three levels of SC

Injury to the epithelial cells due to mastitis can be regarded as the most important reason for calcium reduction. When the microorganisms inter the secretary cells of mammary gland, they can cause infection and damage, therefore normal functions and natural mechanisms of epithelial cells are impaired; for example casein micelle synthesis in Golgi apparatus deteriorates significantly, consequently casein as the main carrier of calcium is lost largely. In fact, as the majority of milk calcium is in company with casein, every change in casein synthesis mechanism might influence the calcium content (Harding 1996). Another mechanism that might be affected by mastitis is the membrane transport mechanism which transports some components namely calcium and casein micelles through membrane of epithelial cells into the milk.

Citrates and phosphates are other groups of components which decrease in milk during mastitis. In aqua phase, calcium exists in the forms of ionized calcium (free ion) or stable complex largely with citrate (trivalent form of citrate) and to a lesser extent with inorganic phosphate (combination of $H_2PO_4^-$ and HPO_4^{2-}) and chloride (Neville and Watters, 1983; Varnam and Sutherrland, 1996). Therefore reduction of these two compounds which are calcium carriers can influence calcium content of milk.

Lesser part of colloidal calcium binds with α-lactalbomine. β-lactoglobulin can also bind with calcium and Magnesium. During mastitis the milk soluble proteins with blood origin increases, while α-lactalbomine and β-lactoglobulin are decreased. These two whey proteins are rather resistant to proteolysis; therefore the reduction is related to the deficiencies in synthesis functions of injured epithelial cells, on the other hand they might move from milk into the extracellular space.

-Milk phosphorus content

The results showed a significant reduction (α=0.01) of phosphorus content in milk by elevating SC level (Figure 2). It seems 84% of the changes in milk phosphorus is due to the increased SC level (R^2 = 0.84) according to the linear regression diagram (Diagram 2).

Phosphate and calcium are recognized as the structural units of casein micelle and play important roles in binding caseins to each other to make a micelle. About half of phosphate in milk is associated with casein micelles. As noted with mastitis incidence and increased SC level, casein content of milk is decreased significantly due to deficiencies in synthesis and secretion functions of epithelial cells, and increased permeability of the blood-milk barrier. Phosphorus content is decreased as the consequence of reduction in the concentration of casein in mastitic milk.

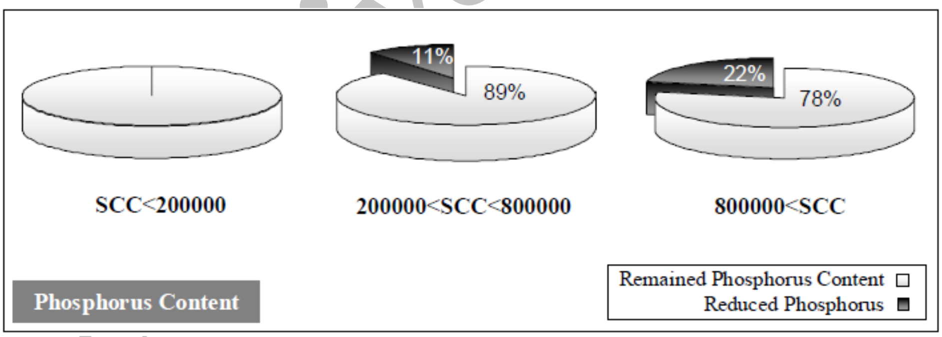

Fig. 2. The percentage of phosphorus content reduction in milk samples in three levels of SC

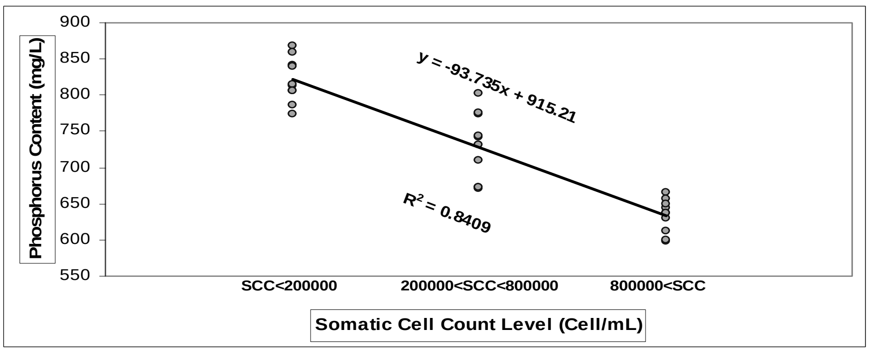

Diagram 2. The linear regression of SC level effect on phosphorus content of milk samples

Phosphate is a by-product of lactose synthesis in secretory cells; the rate of this mechanism falls during mastitis, hence the rate of phosphate production in damaged epithelial cells is decreased (Jensen, 1995).

In addition to phosphate synthesis deficiencies, movement of phosphate into the milk across the secretory cell membrane is not performed as occurs in normal situation, because of injury to these cells; resulting in insufficient phosphate content in sub-clinical mastitic milk and on the other hand the osmotic pressure is changed due to increased permeability of secretory cell membrane and the blood-milk barrier, therefore ionic equilibrium is impaired, thus some components and probably phosphates move from milk into the blood stream or extracellular space. In general, reasons of phosphorus reduction in milk by increased SC level might be described as reduced phosphate synthesis, deficiencies of the membrane transport mechanisms and changes in osmotic pressure.

-Calcium, phosphorus Ratio

With increasing SC level, Ca/P ratio declines (Figure 3). This ratio was significantly different in all three levels of SC (α=0.01) and approximately 72% of this reduction (Diagram 3) is related to the increased SC level in milk (R^2 = 0.72).

Nutritionists recommend drinking four glasses of milk daily for avoiding osteoporosis (Jensen, 1995; Walstra and Wouters, 2006). The balance of calcium and phosphate and their ratio in milk is important from nutritional (optimal calcium intake) and processing aspects. The ratio in milk is between 1 and 1.5 and if the figure is approaching 1.5 calcium intake in the body and milk nutritional properties are improved. Mastitis incident can affect human health by decreasing the amount of calcium and Ca/P ratio in milk. If milk is derived from cows with sub-clinical mastitis and is mixed with other milks, such quality milk might not supply the calcium requirements of human body in terms of both quality and quantity. The reduced Ca/P ratio might also affect the milk final products.

-pH and titratable acidity

In the present study the average pH and TA of milk with low SC level were 6.65 and 15.2^{o}D respectively. Normal milk shows a TA of 14-17^{o}D and a pH of 6.4-6.7 (Early 1998 and Petersen and Gregory 2006). By increasing SC level, pH was increased and TA was reduced. Significant differences in milk pH were observed between low and medium SC levels (α=0.05) as well as medium and high SC levels (α=0.01). A significant increase in TA was found between low and high SC levels (α=0.05). 62% and 85% of changes in pH and TA respectively were related to the increase in SC level, according to R^2 factor in linear regression diagram (Diagrams 4 and 5).

The followings are the reasons for the increase in pH and decrease in TA which are related to the increase in SC levels (Harmon, 1994):

- changes of milk ionic equilibrium as a result of mammary tissue injury and damage to epithelial secretory cells
- increased permeability of the blood-milk barrier in infected quarters and abnormal transference of some compounds from blood into milk and vice versa.

Changes of ionic equilibrium often due to increased amounts of sodium and chloride and reduced potassium ion concentrations in mastitic milk have been reported as the most important reason (Early, 1998). The increased chloride might reduce TA by decreasing the amount of free hydrogen ions in the milk, also the decreased amounts of citrate in the milk, which flows from milk into the blood stream during the disease, can be notable (Petersen and Gregory, 2006).

As observed with mastitis incidence and casein deterioration, the amount of phosphate is reduced and the balance between soluble phosphate and casein in milk is changed. On the other hand some whey proteins such as imonoglobulins (rich in lysine, which is an alkali amino acid) flow into the milk during infection. The normal pH of blood is about 7.3 which is slightly alkaline, and as the SC levels is increased in milk, the pH is changed towards a more alkaline condition due to the diffusion of sodium bicarbonate into the milk from the blood stream (Petersen and Gregory, 2006).

- *Pearson correlation*

Correlation analysis was carried out based on Pearson coefficient. Positive correlation between the two data means those two data increase or decrease together. Thus increased SC level could decrease Ca and P contents, Ca/P ratio and TA, while pH values decreased.

The highest and lowest correlations were observed respectively between titratable acidity and SC level and between pH and SC level (Table 2).

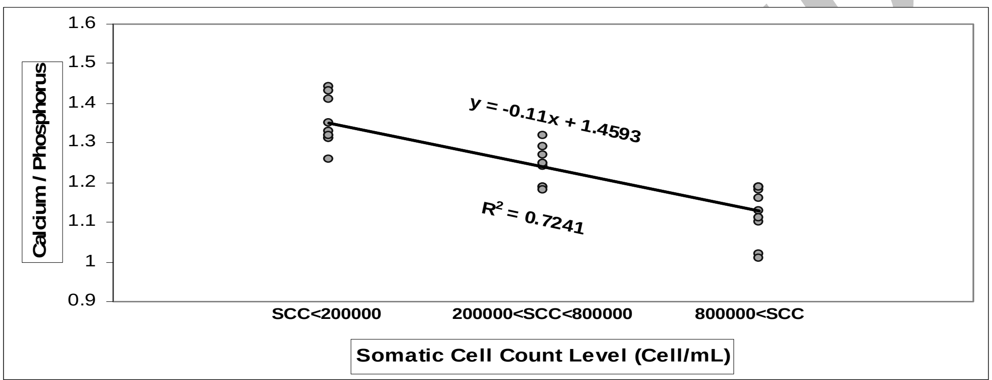

Diagram 3. The linear regression of SC level effect on Ca/P content of milk samples

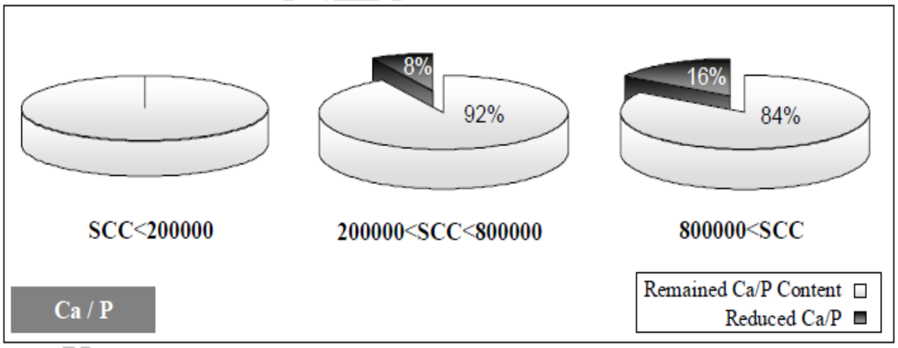

Fig. 3. The percentage of Ca/P content reduction in milk samples in three levels of SC

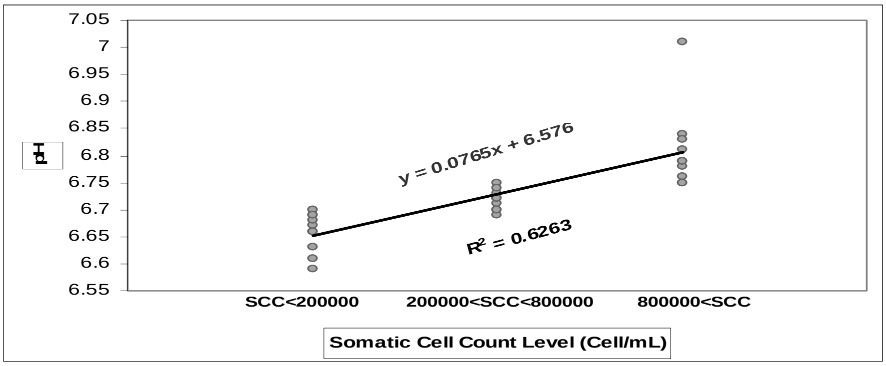

Diagram 4. The linear regression of SC level effect on pH of raw milk samples

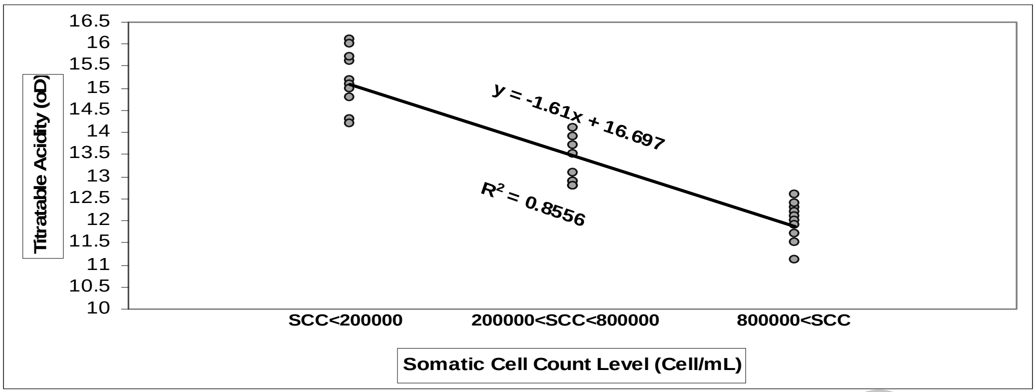

Diagram 5. The linear regression of SC level effect on titratable acidity of raw milk samples

Table 2. Pearson correlation matrix for evaluation of test data correlation in three levels of SC and whole levels

	SCC	pH	TA	Ca	P	Ca/P
SCC	1					
pH	0.727	1				
	0.729					
	0.811					
	0.811					
TA	-0.913	-0.773	1			
	-0.897	**-0.588**				
	-0.942	-0.856				
	-0.972	-0.864				
Ca	-0.769	-0.833	**0.658**	1		
	-0.841	**-0.657**	**0.652**			
	-0.902	**-0.705**	0.837			
	-0.932	-0.868	0.943			
P	-0.805	-0.792	**0.697**	0.978	1	
	-0.860	**-0.653**	**0.696**	0.904		
	-0.809	**-0.563**	**0.630**	0.915		
	-0.923	-0.839	0.940	0.909		
Ca/P	-0.735	-0.814	**0.623**	0.986	0.936	1
	-0.778	**-0.622**	**0.549**	0.963	0.907	
	-0.886	-0.735	0.897	0.970	0.789	
	-0.895	-0.896	0.914	0.980	0.947	

Note: In this table bold numbers show a significant correlation with 95% confidence (r = 0.05) and underline numbers refer to a significant correlation with 99% confidence (r = 0.01)

First row: low SC level, second row: medium SC level, third row: high SC level; and forth row: whole levels in general.

Conclusion

As observed before, increased SC level and mastitis incidence could reduce calcium and phosphorus content, and Ca/P ratio. This effect was observed even in sub-clinical mastitis that disease symptoms and milk abnormalities are not visible. In a farm for every case of clinical mastitis, there are 20-40 times as many cases of sub-clinical mastitis; therefore the loose of calcium content in sub-clinical cases might be regarded as one of the important matters and problems.

The results of the present study showed that the amount of calcium in raw milk, which is the most important milk mineral, significantly depends on good farming practices. To supply the nutritional requirements of vulnerable groups of society, namely pregnant and nursing mothers, children and people with calcium deficiency symptoms, such as osteoporosis patients seriously need to concentrate more attention to dairy practices on farm level. If sub-clinical mastitis is monitored and milk is ranked in the first stages of production, a lot of nutritional problems may be solved. Therefore dairy industry should monitor the SC count frequently. Therefore processing factories can decide more precisely the kind of processing and production according to milk quality, understanding that the production of some dairy products needs critical conditions (such as cheese). Consequently production of high quality dairy products with normal nutritional value cannot be expected without milk grading in farm level and mastitis prevention and controling.

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