Revista Científica, FCV-LUZ / Vol. XXIV, Nº 5, 408 - 413, 2014

EFFECT OF SUBCLINICAL MASTITIS ON THE PHYSICOCHEMICAL QUALITY OF BOVINE MILK

Efecto de la mastitis subclínica sobre la calidad físico-química de la leche bovina

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RESUMEN

La mastitis bovina puede ser clasificada como clínica y subclínica, de acuerdo a la presencia o ausencia de signos clínicos. En ambos casos, hay un incremento de las células somáticas (CS), siendo superior en la mastitis clínica. El propósito de este estudio fue determinar el efecto de la mastitis subclínica sobre la calidad físico-química de la leche en sistemas de ganado doble propósito (DP). Para este estudio se utilizó un muestreo no probabilístico en seis empresas de ganado DP en el municipio de Montería, Córdoba (Colombia). Mediante el test de California para mastitis (TCM) fueron seleccionados los cuartos evaluados como TCM 3 al tomar las muestras y cuartos evaluados como TCM 0, en la misma vaca como control. Las muestras fueron colectadas asépticamente y se guardaron refrigeradas hasta su procesamiento. El análisis físico-químico fue determinado mediante el equipo Biolac 60. La determinación de caseína fue realizada por espectofotometría y el conteo de CS mediante un contador de células óptico portátil. Para la evaluación de las variables físico-químicas y el recuento de CS se agruparon dentro de cuatros fases (0-2 meses, 2-4 meses, 4-6 meses y mayores a 6 meses de lactancia). La leche, con un conteo de CS inferior a 250.000 fueron consideradas sin mastitis subclínica, y con mastitis, las que presentaron conteos iguales o superiores a 250.000 CS/mL. Los promedios para proteína total para leches con alto y bajo conteo de células somáticas fueron 2,93 ± 0,13 y 3,12 ± 0,13, respectivamente. Para el porcentaje de grasa, los promedios fueron 3,36 ± 0,29 para leches con alto conteo de CS y 3,70 ± 0,46 para leches con bajo conteo de CS. En términos generales, las leches con altos conteos de CS, los componentes químicos disminuyeron significativamente (P≤0,05) en comparación con las células de recuento bajo.

Palabras clave: Calidad de la leche, células somáticas, mastitis subclínica.

ABSTRACT

The bovine mastitis can be classified into clinical and subclinical, according to presence or absence of clinical signs. In both cases there is an increase of somatic cells (SC) being higher for clinical mastitis. The purpose of this study was to determine the effect of subclinical mastitis on physical and chemical milk components in dual purpose systems (DP). Using a non-probabilistic sampling in six DP livestock enterprises in Monteria, Cordoba (Colombia), a cross-sectional study was implemented. By California mastitis test (CMT) were selected quarters evaluated as CMT 3 to take samples, and quarters evaluated as CMT 0, in the same cow, for control. The samples were collected aseptically and were kept refrigerated until processing. The physicochemical analysis was determined by Biolac 60 equipment. The determination of casein was done by spectrophotometry, and SC count by an optical and portable cell counter. The evaluation of the physicochemical variables and SC count were grouped into four phases (0-2 months, 2-4 months, 4-6 months and more of 6 months of lactation). Milk with cell count less than 250,000 was defined as without subclinical mastitis and with subclinical mastitis when cell count was greater than or equal to 250,000 SC/mL. The averages for total protein for milk with high and low SC counts were 2.93 ± 0.13 and 3.12 ± 0.13, respectively. For fat percentage, averages were 3.36 ± 0.29 for high count milks SC

Recibido: 03 / 09 / 2013 . Aceptado: 18 / 06 / 2014.

and 3.70 ± 0.46 for milk with low count of SC. Overall, milk with high counts of SC, the chemicals components decreased significantly (P≤0.05) compared to the low count of cells.

Key words: Quality milk, subclinical mastitis, somatic cells.

INTRODUCTION

Mastitis is defined as any inflammation of the mammary gland in response to various infectious agents or non-infectious etiology [34]. The mastitis by the presence or absence of clinical signs can be classified into clinical and subclinical [36]. In both cases, there is an increase of somatic cell (SC) [5].

Invading pathogens activate the immune defense in the udder, is a complex biological process which involves not only resident immune cells and recruited but also mammary epithelial and endothelial cells. The result is the increased number of polymorphonuclear (PMN) by recruitment and epithelial desquamation of lactiferous ducts and udder cistern [15].

Somatic cell count (SCC) is used as indicator of udder health in the cow (*Bos primigenius taurus y Bos primigenius indicus*), sheep (*Ovis aries*) and goat (*Capra hircus*) [35, 36]. In milk from quarters without subclinical mastitis (WSM), the cellularity is low, but in milk from quarters with subclinical (SM) or clinical mastitis (CM), the PMN increase according to the severity of inflammation [19].

Besides the decreased volume of milk produced as a result of mastitis [14], the physical and chemical composition of milk changes, such as, the casein, major protein, decreases as a consequence of infection and the whey proteins (lactalbumin and lactoglobulin) are increased by augmented vascular permeability, reduces the stability of the milk to heat by coagulation or flocculation during pasteurization and ultrapasteurization processes, also there is a decreased calcium in milk; factors affecting milk yield in the production of cheese [12], potassium predominant mineral in milk, declines and the concentrations of sodium and chloride ions rise and increases the electrical conductivity. The lactose content is reduced. Plasmin and other proteolytic enzymes, derived from SC, cause lysis of casein in the udder; proteolytic activity of plasmin is increased more than two times during mastitis [26]. The deterioration of milk continues during the storage and may have casein proteolysis due to presence of proteolytic enzymes [9]. The purpose of this study was to determine the effect of subclinical mastitis on physical and chemicals components of milk in dual purpose systems in the Colombian tropical low.

MATERIALS AND METHODS

Type of study

Using a non-probabilistic sampling and convenience, in six Dual-Purpose (DP) cattle enterprises, located in Monteria Municipality, Cordoba (Colombia) a cross-sectional study was implemented [6].

Animals and type of management

For this study, cows from crosses between *Bos taurus* x *Bos indicus* were used. The cows had between two and eight calving. The animal feeding was based angleton (*Dichantium aristatum*) pastures and were supplied mainly mineralized salt and water *ad libitum*. Milking was done in the morning (at around 5: am) and calf remained with the cows till noon, and then is separated from the mother until the next day. The samplings were taken in the rain season.

California Mastitis Test

California Mastitis Test (CMT) was applied to all cows that on the day of the visit was in milking, according to the proposed by Philpot and Nickerson [36]. In order to compare the SM effect, only were sampled quarters evaluated as CMT 3 (maximum grade of SM and as control, quarters evaluated as CMT 0 (WSM) in the same cow.

Sampling

The samples were taken aseptically, for which, each teat was washed and dried with a disposable towel, subsequently the teat end was disinfected rubbing with cotton swabs soaked with antiseptic alcohol 70% as often as necessary, until the last swab keeps clean. Ten mL of milk were taken in sterile glass screw-cap. A total of 86 samples of milk were collected, 43 cases (CMT SM 3) and 43 control samples. The samples were kept refrigerated in an expanded polystyrene cooler until processing at the Biological Research Institute of the Tropics (IIBT) of the University of Cordoba (Colombia).

Physicochemical analysis of milk

Percentages of total protein, fat and non fat solid (NFS), mineral salts, lactose, cryoscopic point, and density were determined according to the method referenced by International Dairy Federation (IDF) 141 B [25], using an ultrasonic milk analyzer Lac 60 (Boeco, Germany). The samples were mechanically stirred during five min and then processed at 20° C. The determination of casein was done by spectrophotometry, for which a standard curve made with casein 95% purity, using the Biuret method [30, 42], scanning was performed between 540 and 580 nm, showing a maximum absorption lamda peak at 560 nm [27].

Somatic cells determination

The somatic cells count (SCC) was performed with a optical and portable SC counter (DCC, DeLaval International AB, Sweden). The samples were mechanically stirred during five min and with a device cassette-type 60 µL of milk were taken. This device contains capillary impregnated with a fluorescent probe labeled with propidium iodide that binds specifically to cellular DNA. Then the device was placed in the reading camera, where it was exposed to the action of light, resulting in the production of fluorescent signals that were counted [18].

Statistical analysis

For the evaluation of all physicochemical variables and SCC, the milk samples were distributed into four groups: phase 1, 0-2 (0 – 8 weeks) months of lactation; phase 2, of 2-4 (9-16 weeks) months; phase 3, 4-6 (17 -24 weeks) months; and phase 4, 6-8 (25 – 32 weeks) months. For this work was defined as SM free milk, when SCC was less than 250,000 SC/mL, and subclinical mastitis milk with count greater than or equal to 250,000 SC/mL, the literature has used different thresholds to differentiate healthy from unhealthy udders [29]. A value with minimal diagnostic error, 200,000 cells/mL was proposed by Schukken et al. [41], Pyörälä [37] defined that subclinical mastitis can be considered ≥ 200,000 SC/mL without growth in bacterial culture. Caraviello [13] said healthy mammary glands have values < 200,000 CS/mL and higher counts are usually associated with an infection. Khan and Khan [28] counts <100,000-150,000 CS/mL as no SM. Barlow et al. [3], defined and identify cases of SM as milk with ≥ 200,000 CS/mL.

Analysis of each sample were performed by triplicate and obtained an average; arithmetic mean, standard deviation and variance were obtained. For the SCC results were transformed (log transformed) because the data did not show a normal distribution. The dates were analyzed using the SAS statistical software [40].

Ethical aspects

The cows used in this experimentation were handling according to ethical standards established by law 84/1989 of the Republic of Colombia [38]. The ethics committee of the IIBT reviewed, qualified and approved this work, as with low impact.

RESULTS AND DISCUSSION

In the TABLE I, the changes of the physicochemical quality of milk with SM and WSM are presented, in each stage of lactation. The TABLE II, shows the differences of the physicochemical variables of milk with SM and WSM.

A significant difference (P<0.05) was determined when compared all variables included in the study. In each lactation stage, milk from udder with subclinical mastitis has a decrease of chemical and physical compounds concentration compared to milk from udder WSM (TABLE I). This decrease was due to the rise in SC as a result of inflammation which reduces the synthesis of the different milk components and also due to increased vascular permeability, causing an abnormal increase in blood plasma containing enzymes such as proteases, lipase and plasmin, that accelerate the breakdown of protein and milk fat [20, 26,29]. Vinhas et al. [46] concluded that a high SCC directly affects the quality of the milk The density of the milk from udder with SM showed significant difference in relation to milk from udder WSM. Equally, when decrease the synthesis of all chemical components of milk [16], the cryoscopy point increases, which could result in a mistaken presumption of impaired milk with addition of water [22]. The milk density average with low SCC milk was 1,032 g/mL (TABLE II), value previously reported for milk in Colombia [10, 11].

The value of protein percentage was statistically different (P<0.05) in milk from udder with SM compared with milk from udder WSM. Milk from cows with mastitis has a reduced level of casein, but lactalbumin and lactoglobulin levels increase [26]. Schukken et al. [41] observed that when lowered the SCC, the fat and lactose increased but this increase was lower for protein. In high SCC milks a decrease of protein be-

TABLE I

AVERAGE PHYSICOCHEMICAL QUALITY OF MILK WITH AND WITHOUT SUBCLINICAL MASTITIS IN EACH STAGES OF LACTATION IN COWS DUAL PURPOSE IN MONTERIA, CORDOBA (COLOMBIA)

	1 Phase lactation		2 Phase lactation		3 Phase lactation		4 Phase lactation	
Properties	↑ SCC	↓ SCC	↑ SCC	↓ SCC	↑ SCC	↓ SCC	↑ SCC	↓ SCC
Total protein (%)	3.00[a]	3.09[b]	2.89[a]	3.08[b]	3.00[a]	3.25[b]	2.81[a]	3.11[b]
Caseín (%)	2.21[a]	2.41[b]	2.27[a]	2.44[b]	2.23[a]	2.57[b]	2.11[a]	2.46[b]
Fat (%)	3.46[a]	3.932[b]	3.40[a]	3.64[a]	3.35[a]	3.76[b]	3.24[a]	3.76[b]
Lactose (%)	4.29[a]	4.69[b]	4.13[a]	4.68[b]	4.24[a]	4.77[b]	4.12[a]	4.74[b]
Non fat solids (%)	7.65[a]	8.40[b]	7.62[a]	8.57[b]	7.54[a]	8.59[b]	7.27[a]	8.73[b]
Minerals (%)	0.742[a]	0.700[b]	0.694[a]	0.696[b]	0.740[a]	0.692[b]	0.760[a]	0.687[b]
Totals solids (%)	11.11[a]	12.33[b]	11.01[a]	12.21[b]	10.88[a]	12.35[b]	10.51[a]	12.50[b]
Densityd (gr/mL)	1.028[a]	1.033[b]	1.029[a]	1.033[b]	1.029[a]	1.032[b]	1.029[a]	1.032[b]
Cryoscopic point	0.521[a]	0.548[b]	0.512[a]	0.540[b]	0.522[a]	0.552[b]	0.517[a]	0.550[b]
SCC (SC/mL)	474,061[a]	79,818[b]	56,1424[a]	70,697[b]	91,3545[a]	37,048[b]	2,411,182[a]	39,812[b]

[a]For the same stage of lactation different letters in the same row are statistically different (P<0.05).
↓ SCC: < 250,000 SC/mL; ↑ SCC: ≥ 250,000 SC/mL.

TABLE II
AVERAGE PHYSICOCHEMICAL QUALITY OF MILK WITH AND WITHOUT SUBCLINICAL MASTITIS ACCORDING IN COWS DUAL PURPOSE IN MONTERIA, CORDOBA (COLOMBIA)

Properties	↑ SCC	↓ SCC
Total protein (%)	2.93 ± 0.13[a]	3.12 ± 0.13[b]
Casein (%)	2.21 ± 0.13[a]	2.47 ± 0.12[b]
Fat (%)	3.36 ± 0.29[a]	3.70 ± 0.46[b]
Lactose (%)	4.19 ± 0.21[a]	4.76 ± 0.15[b]
Minerals (%)	0.75 ± 0.02[a]	0.70 ± 0.02[b]
Totals solids (%)	10.88 ± 0.49[a]	12.32 ± 0.56[b]
Density (gr/mL)	1.029 ± 0.001[a]	1.032 ± 0.001[b]
Crioscopia point	- 0.52 ± 0.01 [a]	- 0.55 ± 0.01[b]
SCC (SC/mL)	1,090,053 ± 954,793[a]	54,756 ± 54,756[b]

[a]For the same stage of lactation different letters in the same row are statistically different (P<0.05).
↓ SCC: < 250,000 SC/mL; ↑ SCC: ≥ 250,000 SC/mL.

tween 6 to 8% was reported [21]. The degree of mastitis diminishes the values of protein in buffalo (*Bubalus bubalis*) milk [45]. Phenomena of proteolysis and epithelial permeability increase the levels of plasmin and other proteases from SC leading to degradation of casein [1]. Batavani et al. [4] found that the content of albumin in milk in SM milks was significantly higher compared with milk from healthy quarters. Equally, Andreatta et al. [2] found significant differences (P<0.05) for protein in milk with high SCC.

Comparing the percentage of casein between samples analyzed it was found that the value was statistically different in milk with SM compared with milk WSM at each stage of lactation. This significant difference for mastitis free milk may be due to rise plasmin, proteolytic enzyme that lyses the casein [26]. Haenlein et al. [23] concluded that when the CCS was >500,000 SC/mL there was a decrease on casein and that this was greater when >1,000,000 SCC.

It was determined that the fat percentage has a difference statistically significant (P<0.05) between milk with SM and WSM, unlike of Roman et al. [39] in Venezuela, who found no significant differences in the percentage of milk fat of 82,000 and 325,560 SC/mL. Bernal et al. [7], identified a highly significant correlation between total solids and fat (r = 0.7; P<0.05). Bonfoh et al. [8], in Mali showed that the percentage of fat was influenced by the supplement feeding, race, age, breastfeeding and SM. Ullah et al. [45] determined that the severity of mastitis decreases fat values from 5.01 ± 0.19%, maximum content of buffalo milk negative for subclinical mastitis, to 4.91 ± 0.17% and 4.39 ± 0.15% in SM. Leitner et al. [31] showed that bacterial infection significantly affected the concentration of fat and protein in milk.

These significant differences (P<0.05) of the chemical parameters of the milk as: fat percentage, lactose percentage, nonfat solids percentage, minerals percentage, total solid percentage, may be due to an increase in SC as a result of inflammation which reduces the synthesis of the different components of milk and increased vascular permeability. This causes an abnormal increase in blood plasma containing enzymes such as proteases, lipase and plasmin enzymes that accelerate the breakdown of protein and milk fat [20, 26, 29]. In this study, when compare the variations of SCC and protein, between the different stages of lactation, according to increases of the phase, increases SCC and decreases protein percentage.

It was found that at each stage of lactation, the difference between the concentration of lactose in milk with SM and milk WSM was statistically significant (P<0.05) (TABLE I). Harmon [24] established that the mastitis reduce lactose concentration between 5 and 20%. Bonfoh et al. [8] found that the concentration of lactose did not differ significantly between herds and cows but there was significant difference in accordance with SCC. The rise in the SCC and the changes in milk composition were more marked in the quarter affected by major pathogens (*Staphylococcus aureus, Streptococcus agalactiae*, other species of *Streptococcus*, and *Escherichia coli*) that minor pathogens (coagulase-negative *Staphylococcus* and *Corynebacterium* spp.), indicating that the severity of inflammation was associated with the type of pathogen [1]. Sharif et al. [43] determined a decrease in lactose content according to the SCC increase in buffalo milk. Dangh and Anand [17] confirmed that high SCC is characteristic of an infection and this resulted in a reduction in lactose. Sharif et al. [44] explained that as increases the severity of mastitis and SCC in buffalo milk, the lactose content decreased significantly. Ogola et al. [33], claim that a high prevalence of SM is a major constraint in the production of quality milk. These decreases in the concentration of milk components are due to the reduction of synthetic activity of alveolar tissue [21, 32].

When comparing the physicochemical variables of milk from high and low SCC, at all stages of lactation (TABLE II) was determined that all the variables studied were statistically different (P<0.05).

CONCLUSIONS

In milk from SM was determined that the chemical components of raw milk decreased significantly compared with no SM milk, which affects its quality. Among the physical variables, the cryoscopic point increased as a result of decreased synthesis of all the chemical components of milk and decreased density, which could be misconstrued as an addition of water to milk. The SCC is an excellent tool for diagnosing the health of the mammary glands.

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