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On effects of subclinical mastitis and stage of lactation on milk quality in goats

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ABSTRACT

Bulk milk is the mixture of all the milked udders in a given herd. Nowadays, about 15–40% of the udders in most herds are intramammary infected by different bacteria species, mainly coagulase negative staphylococci. The presences of bacteria in the lumen of the mammary gland induce impairment of milk quality and increase the number of somatic cells. A positive relationship between % casein (casein/total protein) and curd firmness (CF) and negative relationship between lactose, or somatic cell count (SCC) and CF are associated with bacterial infection and with late lactation milk, and therefore with reduction in cheese yield and quality. On the other hand, in milk of goats with intramammary infection, the correlation between the levels of fat, protein, casein and curd yield is minor compared to milk of uninfected animals. Thus, gross milk composition is an insufficient predictor of milk quality for cheese production, since a high percent of the bulk milk originates from subclinically infected glands. Research carried out in the past few years highlighted the effectiveness of lactose as a predictor of milk quality. The correlation between lactose and CF was higher than that for % casein and SCC. Lactose concentration of $\leq$4% is associated with non-coagulating milk and therefore, such milk is unsuitable for making cheese, but still meets the criterion for consumption as pasteurized milk. A model that describes the simultaneous and close association between reduction in lactose concentration and milk yield on the one hand and reductions in lactose concentration and milk quality on the other hand is presented. The physiological and biochemical basis for deterioration of milk quality in subclinically infected and in late lactation animals is reviewed and suggestions to improve the quality of milk produced by farmers and acquired by dairies are presented.

1. Introduction

Goats are among the earliest domesticated farm animals and their milk has been consumed by humans for thousands of years. Goat herding practice dates back to 8000 BC (Colledge et al., 2013) and evidence of using goats milk for making dairy products goes back to ancient Egypt, where remains of pots that has been used to make or store cheese were discovered in Pharaohs burial tombs (Edelstein, 2014). On a global scale, total goat milk production reaches 15,510,411 tons per year, of which 80% are produced in developing countries where goat's milk plays an important role in the livelihood of hundreds of millions of human beings (FAO, 2013; Silanikove et al.,

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2010). In Europe and North America the vast majority of goat's milk is used to make dairy products, most notably cheese (Morand-Fehr et al., 2004). The yield and quality of cheese is crucially depended on milk quality. Milk quality for cheese production is influenced by many factors such as nutrition, genetics, husbandry, which are mostly covered in the publications of this special issue. The most important single factor that determines cheese yield and quality in dairy ruminant (sheep and cows) in general and in goats in particular are the deleterious effects of subclinical mastitis (SCM)(Leitner et al., 2006; Silanikove et al., 2010; Martí-De Olives et al., 2011), bearing in mind that milk from clinically infected goats should not reach the food-chain. Milk produced at the end of lactation also significantly and negatively affects milk quality for cheese production in sheep, cows and goats, and the effect is particularly evident in goats (Leitner et al., 2011a). Both, SCM and end of lactation effects on milk quality are imposed through inducing inflammatory stress on milk components (Leitner et al., 2012). Thus, separation of these effects is confounded and imposes difficulties on the dairy industry for grading milk quality for cheese production (Silanikove et al., 2010). The aim of this review is to critically consider recent advances on how SCM and end of lactation affect milk quality for cheese production and to devise recommendations on how to deal with these conditions.

2. Effects of subclinical mastitis and end of lactation on milk yield and quality

The first step in producing cheese is producing curd. In milk from uninfected glands, curd yield depends mainly the content and subtypes of αS1-, β- and κ-caseins (Hyslop, 2003; Vazquez-Flores et al., 2012; Mestawet et al., 2014), fat level, and protein to fat ratio (Guinee et al., 2007). Phosphoseryl residues on αS1- and β-caseins are bound in the micelle to polyvalent cations, mostly Ca^{+2} ions. Expositions of the phosphoseryl residues due to the activity of clotting enzyme lead to charge neutralization, aggregation, and eventually to precipitation of the curd components. During aggregation, casein forms a fine mesh that entraps the fat globules and leaves the soluble lactose in the whey. Thus, the main components of curd are casein and minerals associated with it, most notably Ca^{+2}, fat and components attached to the milk fat globule membranes, such as fat soluble vitamins.

Cheese is prepared by utilizing a wide range of different microbial cultures. In combination with maturation conditions and maturation period, impressive wide varieties of cheeses, each with unique taste, shape, color, texture and rheological properties are produced worldwide. Recently, it was shown that cheese made from milk of SCM infected glands affected negatively the chemical processes that occur during cheese maturation. Despite the fact that the curd mass of cheese made from milk taken from uninfected glands was equal to the curd mass of cheese made from milk taken from infected glands, the final produced from milk coming from the infected glands had lower yield and quality (Merin et al., 2008; Rovai et al., 2014).

2.1. Experimental models to investigate the effects of SCM

Use of the half-udder model in which a single gland serves as the experimental unit and the contra-lateral gland as a control has been used extensively by our research group for studying the effect of SCM on milk yield and milk quality (Leitner et al., 2004a,b,c, 2006, 2008a,b, 2011a) and was also used by others (Gonzalez-Rodriguez et al., 1995; Martí-De Olives et al., 2013). This experimental model enables to study the physiological basis and quantification of the negative effects of SCM on milk yield and quality with high statistical reliability, even for a relatively small data set of 20–40 animals. The half-udder model proved as an effective tool for isolating the experimental effect from numerous masking effects. The experimental variability arose from significant individual variations between individual animals and is farther complicated by the effects of factors such as, farm management, environmental conditions, age, and stage of lactation. Obviously, variations caused by all of these factors are neutralized when the units of comparison are the two glands of the same animal. An alternative approach based on conventional whole-udder sampling would have required a data set in the order of 100 animals to account for the large above-described source of variability (Leitner et al., 2004b).

However, because of the tendency of the uninfected gland to compensate for reduction in milk yield of the infected gland, and because such compensation is not possible in the event where both glands are infected, the effect of SCM on a whole animal level was found bigger than that obtained by the average effect found with the half-udder model (Leitner et al., 2008a). Thus, for the purpose of quantifying the effect of SCM on a whole herd level, especially in herds with poor hygienity, it is preferable to carry out experiments that include milk sampling of all the goats in a given flock and to combine this information with information gained by the half-udder model (Leitner et al., 2004b, 2007).

2.2. The bacteria involved and etiology of infection

Worldwide, intramammary infection (IMI) in small ruminants (sheep and goats) and large ruminants (cows) is a major cause of economic loss to the dairy industry. Effective control of new IMI cases and, consequently, milk yield, milk quality, including somatic cell counts (SCC) in the milk of dairy animals is aided by an understanding of the pathogens involved, the source of infection and the frequency of spontaneous cures (Lam et al., 1997; Leitner et al., 2007).

In goats, as in sheep and cows, SCM is the prevalent form of mastitis (Bergonier et al., 2003; Leitner et al., 2004b,c, 2007). The prevalence of SCM in small ruminants could be as low as 5% under very good husbandry, but, typically affects 15–40% of the animals in a given flock. On the other hand, annual incidence of clinical mastitis is generally lower than 5–10% (Contreras et al., 2007; Silanikove et al., 2010). Staphylococci: *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) are frequent pathogens isolated from IMI goats (Contreras et al., 1999, 2007; Leitner et al., 2004b). However, CNS, mainly *Staph. caprae*, *Staph. epidermidis*, *Staph. chromogenes* and *Staph. simulans*, comprise the most abundant bacterial isolates, and dominated the bacteria isolated from IMI in goats in almost all flocks tested in different parts of the world (Kalogridou-Vassiliadou et al., 1992; Contreras et al., 1999; Foschino et al., 2002; Moroni et al., 2005; Contreras et al., 2007; Silanikove et al., 2010; Souza et al., 2012).

Characterizing the etiology of bacterial infection is important for understanding the grounds for establishing of infection and devising appropriate treatments to control the contagion. However, very little research has been done to explore the patterns of IMI acquiring in goats. The only study that we are aware of in which the etiology of IMI was systemically analyzed was that of Leitner et al. (2007), which comprised a survey of three goats flocks in Israel. It was found that ~15% of the yearling does were already infected with bacteria when they joined the flock, whereas ~8% of the goats that dried-off with no infection returned with new IMIs. Virtually, none of the goats acquired infection during lactation. Thus, the etiology of IMI in goats was found to be very similar to that in dairy cows (Leitner et al., 2008b), which leads us to believe that this study represent a genuine description of IMI spread in goats. The study of Leitner et al. (2007) defied a common view that prevailed before 2007, but was never sustained by experimental evidences, that goats acquire bacterial infection during milking throughout lactation. The results suggest that as in cows, preventive measures against acquiring IMI need to be concentrated on applying effective dry-off treatment and on preventing the stress of parturition, or in early identification of animals that would resist such stress (Leitner et al., 2008b). However, we are aware of only few efforts to use antibiotic for dry-off treatment in goats and the results regarding the efficiencies of those treatments are contradictive (Poutrel et al., 1997; Mavrogianni et al., 2004; Contreras et al., 2007; Leitner et al., 2007).

2.3. Use of SCC for predicting losses of milk and curd yield

IMI infection with CNS induced inflammatory response, which is reflected by increased SCC (Contreras et al., 1999; Leitner et al., 2004a). The inflammatory response is associated with reduction in milk yield in the infected glands compared with that of the uninfected ones, as also found in sheep and cows (Leitner et al., 2004a, 2006, 2011a; Martí-De Olives et al., 2013; Fig. 1). In sheep the reduction in milk yield is much more significant when both glands are infected than when only one, because the contra lateral gland compensate for the reduction and in goats the compensation is even higher (Leitner et al., 2008a).

Extensive field studies in France (Baudry et al., 1997), Spain (Contreras et al., 2007), Israel (Leitner et al., 2007) and the USA (Barrón-Bravo et al., 2013) leaves no doubt that on the farm level, increased SCC associated with IMI reduce milk yield in comparison to farms with low SCC. The ability of SCC to predict reduction in milk and curd yield is considered below.

By applying the half-udder model, it was shown that curd yield was significantly lower in infected halves in comparison to uninfected ones, although casein contents were almost equal in the two glands (Leitner et al., 2004a,b, 2011a). Similar negative effects of SCM on curd and cheese, which are not associated with casein content, were also reported for cow milk (Auldist and Hubble, 1998; Leitner et al., 2006; Merin et al., 2008). Thus, these data indicate that knowledge of the gross casein and fat contents in the milk is insufficient for predicting curd yield in milk affected by the presence of bacteria in the mammary gland lumen and the potential biochemical reasons for that are discussed below.

Although there is a clear trend for inverse relationship between high SCC and milk yield, or milk quality, using SCC level for such predictions is not simple. SCC are composed mainly of different types of leukocytes, neutrophils, macrophage and T-cells, in addition to epithelial cells (Leitner et al., 2011b, 2012). Whereas, any kind of bacterial infection will result in increased SCC in milk, the level of the increase of SCC sub-types is unique to the kind of bacteria, time after infection occurrence and species involved, because it depends on specific interactions between the invader and the host immune system (Leitner et al., 2006, 2012). Some bacteria species, such as *Streptococcus dysgalactiae* and *Escherichia coli* are much more devastating in terms of their effect on milk quality. However, impairment of milk quality in cows infected by those bacteria was not well predicted by an increase in SCC. Deterioration of milk quality in SCM cows was found to be directly related to liberation of peptides with anti-clotting properties from casein, to changes in casein micelle that impaired clotting (Merin et al., 2008; Fleminger et al., 2011, 2013) and to the level of imposition of oxidative stress on milk proteins (Silanikove et al., 2007). The above-described effects on milk quality were studied in bovine milk. A recent study shows that the same effects are relevant in the case of goats, though goat milk quality is less affected by SCM in comparison to bovines (Silanikove et al., 2014a).

However, despite these limitations, and in view of the ease of measuring SCC routinely, it seems that it still possible to use SCC as criteria for milk quality as elaborated below. A scheme to grade milk according to the level of SCC in goats was proposed by Leitner et al. (2008a). In deriving the scheme, we took advantage of the following factors: (i) prevalence of CNS as the causative agent of subclinical IMI in goats, (ii) subtypes of CNS interact similarly with the immune system and thus cause similar increase of SCC, and (iii) information gained from series of studies on infection within individual animals of both the herd and the gland level. Care was taken that when data from whole animals were used it did not relate to the first week of lactation and to data gathered after 180 days in milk. These restrictions were applied in order to reduce as much as possible the confounding effects discussed above, particularly the effect of stage of lactation. The recommendations to grade goat's milk were as follow:

Grade A: SCC < 840,000 cells/mL, associated with subclinical bacterial infection of up to 25% of goats in the herd, milk loss of up to 0.8% and curd loss of up to 3.3%.
Grade B: SCC > 840,000 and lower than 1,200,000 cells/mL associated with subclinical bacterial infection of up to 50% of goats in the herd, milk loss of up to 1.5% and curd loss of up to 6.5%.

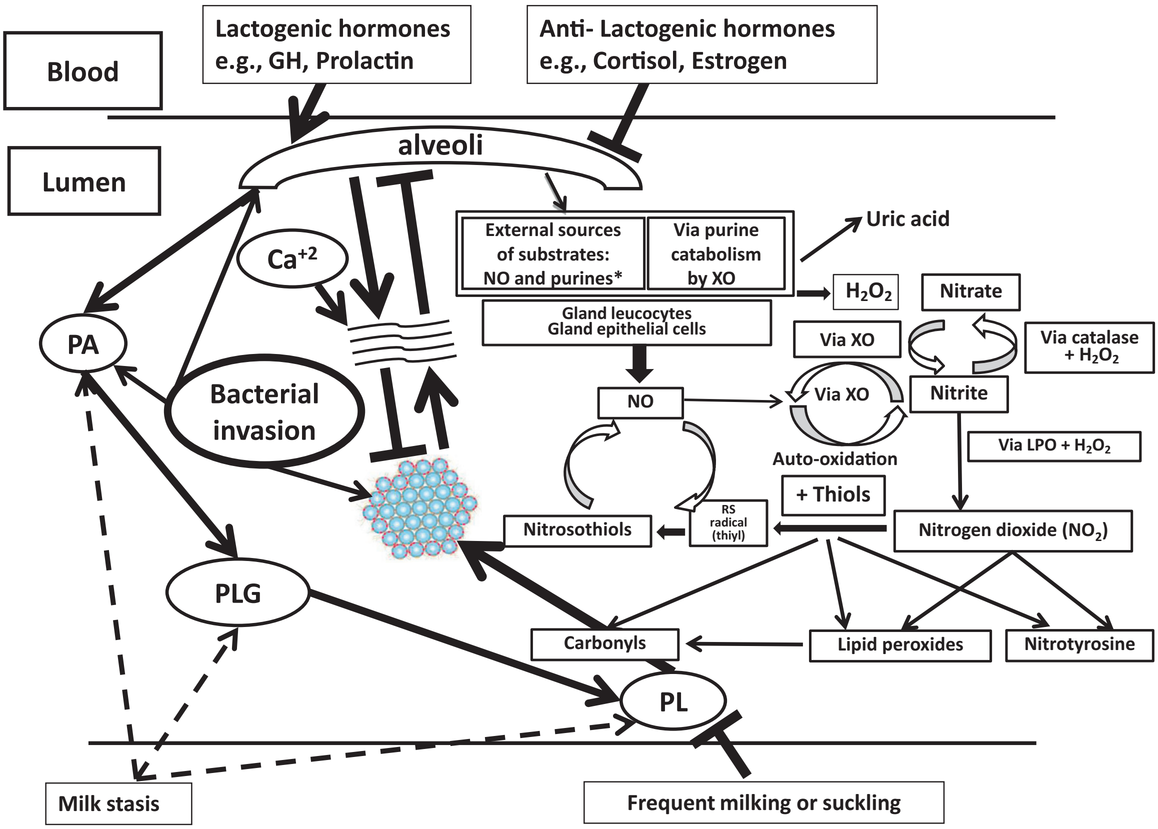

Fig. 1. Schematic model that describes the simultaneous activation of the plasmin (PL) system and the nitric oxide NO-derived cycle in subclinically infected mammary glands. The increased activity of PL causes release of peptides from the casein micelles, which in turn down-regulates milk secretion and casein micelles clotting (Leitner et al., 2011a). The release of peptides that are rich in phosphates impairs the coagulation of milk by reducing Ca^{+2} availability (Fleminger et al., 2013). In parallel, the pro-inflammatory peptides released by PL up-regulates the NO-cycle rate in milk (Silanikove et al., 2005, 2009). The increased release of NO into milk is associated with up-regulation of formation of bactericide radical (nitric dioxide), which is associated with formation of nitrotyrosine, carbonyls and lipid peroxide (Silanikove et al., 2005, 2009).[2] Hydrogen peroxide plays important role in the NO-cycle by its use as substrate for LPO in forming nitric dioxide and as substrate for CAT in conversion of nitrite to nitrate. The later reaction is the main mechanism, which restrain the NO-cycle in milk. The source of hydroxen peroxide in milk is oxidation xanthine and hypoxanthine by XO, which results in accumulation of uric acid as the end product of the xanthenes oxidation (Silanikove et al., 2005, 2009, 2012). The increased content of oxidized components in milk most likely increases their susceptibility to proteolysis of milk proteins (Leitner et al., 2006). Explanation of symbols and abbreviations used in the figure: Casein-derived active peptides: , casein micelle: , CAT = catalase, LPO = lactoperoxidase, NO = nitric oxide; PA = plasminogen activator, PL = plasmin, PLG = plasminogen, XO = xanthine oxidoreductase.

Grade C: SCC > 1,600,000 and lower than 3,500,000 cells/mL associated with subclinical bacterial infection of up to 75% of the goats in the herd, milk loss of up to 2.3% and curd loss of up to 9.8%.

As far as we are aware of, this is the only scheme that provides recommendation on how to grade goat's milk for industrial use. The data clearly indicate that the loss of curd yield is greater than the losses of milk yield.

A recent wide survey covered data from several years which was based on monitoring SCC and milk yield in the USA. It concluded that high SCC may be associated with up to 30% reduction of milk yield in comparison with milk yield in goats with low SCC (Barrón-Bravo et al., 2013). However, in the latter study no effort was made to isolate the effect of IMI, thus it may reflect the above-discussed confounding effects. In particular, the combination of end of lactation and IMI is expected to be influential and may explain the great gap between the two latter mentioned predictions.

According to the propositions made by Leitner et al. (2008a), milk with >3,500,000 cells/mL should not be accepted for marketing because of: (i) The high probability that such milk will contain pathogens and toxins, (ii) Its poor industrial quality, very low or complete absence of curdling, and (iii) The potential formation of toxic radical substances in the milk (Silanikove et al., 2012, 2014a).

3. Physiological and biochemical basis for the effects of SCM and late lactation on milk yield and quality

3.1. The effect of SCM

SCM is typically a chronic situation, which represents a compromise between the inability of the host to eradicate the bacteria and restriction of the pathogen presence to the mammary gland where it does not impose threatening challenge to the life of the organism (Leitner et al., 2011a). The immunological interaction of the host with CNS that cause subclinical IMI infection in goats in comparison to sheep and cows was recently described (Leitner et al., 2012).

The physiological basis for the decline in quality of milk from IMI glands was extensively studied by our group (Shamay et al., 2002, 2003; Leitner et al., 2004a,b, 2007, 2008a, 2011a, 2012; Silanikove et al., 2005, 2006, 2009, 2010, 2014a,b). These studies, in agreement with previous ones, have highlighted the role of the plasmin system in down regulation of milk yield and breaking casein micelles during IMI (Bastian and Brown, 1996; Politis, 1996). An updated version of a negative-feedback regulation based on the plasmin system activity is presented in Fig. 1. According to this model, enzymatic hydrolysis of casein by plasmin liberates peptides that serve as local regulators of mammary gland functions. In particular, a peptide that is formed by the activity of plasmin on β-casein *f*(1–28), which down-regulates milk secretion in cows and goats. β-casein *f*(1–28) reduces the output of lactose and other osmotic components from the alveoli into the gland lumen. This insight accounts for the coordination between the acute reductions in milk yield and milk quality in response to subclinical infection. Casein derived peptides cause the disruption of the tight junctions between mammary epithelial cells (Shamay et al., 2002, 2003; Silanikove et al., 2013a). Casein-derived peptides are chemotactic substances that induce flow of leukocytes, mainly neutrophils, into the mammary gland lumen and explain the increase in SCC (Leitner et al., 2012). The inflammation is associated with surge of nitric oxide that causes nitrosative stress to milk organic components and contribute further to the deterioration of milk quality (Silanikove et al., 2014b, Fig. 1). Plasmin derived peptides also reduce the coagulation of milk (Merin et al., 2008; Fleminger et al., 2011, 2013). The evolutionary physiological basis that underlines the reduction in milk clotting parameters under mastitic conditions is most likely associated with prevention of formation of coagulates that might obstruct the evacuation of secretions from the mammary glands and thus, in turn, lead to complications such as necrosis and uncontrolled inflammation (Leitner et al., 2011a,b).

3.2. The effect of late lactation

Variations in milk yield and composition in goats are also affected by within and between breed variations, parity, estrous, environmental effects and management practices (Silanikove, 2000; McDougall and Voermans, 2002; Leitner et al., 2007; Stuhr and Aulrich, 2010; Barrón-Bravo et al., 2013). However, secondary to the important effect of SCM on milk quality is the effect of end of lactation (Leitner et al., 2011a): In sheep, goats and cows, end of lactation modifies the casein micelles structure and salt equilibrium; consequently, its technological, physico-chemical properties and cheese quality (Lucey and Fox, 1992; Fedaku et al., 2005; Leitner et al., 2011a,b). The simultaneous reduction in milk yield and milk quality is notably greater in goats than in cows and sheep (Leitner et al., 2011a). A unique feature of end of lactation effect in goats is the marked elevation of SCC in milk coming from glands free of bacteria towards the end of lactation, which is associated with elevation of additional markers of inflammation (Rota et al., 1993; Wilson et al., 1995; Leitner et al., 2012; Persson et al., 2014; Silanikove et al., 2014a). The SCC in dairy cows milk is the most important criterion used to grade milk according to its hygienic properties in Western countries. However, in dairy goats the use of this criterion is confounded due to the increase in SCC toward the end of lactation, irrespective of bacterial infection. Thus, a solution to this problem is necessary before applying quality schemes based on SCC for goat milk. One possibility that was suggested is to use milk with high SCC of late lactating goats only for drinking, using bacterial isolation as the major hygienic quality criteria (Silanikove et al., 2010). Applying this solution would be quite simple in flocks in which the goats are dried-off at about the same period. In multi-seasonal breeding programs, applying this solution is much more difficult, as it will necessitate storing separately the milk from late lactating goats. The difficulty in applying the above-mentioned solution should be considered against the recent finding that the milk from late-lactating goats is practically worthless for cheese making (Leitner et al., 2011a).

Consistent with the model presented in Fig. 1, end of lactation in goats is characterized by a particular sharp increase in plasmin activity (Fantuz et al., 2001; Leitner et al., 2004a,b, 2011a) and consequently with accelerated casein breakdown. As discussed above, casein hydrolysates simultaneously reduce milk yield and impair milk clotting. Thus, the larger increase in casein degradation in late lactation in goats in comparison to sheep and cows is explained by the more rigorous immune response and consequential more intense activation of the plasmin system (Leitner et al., 2012; Fig. 1).

The inflammatory response at the end of lactation may be interpreted as a pre-adaptive response to the forthcoming involution stage (Leitner et al., 2011a, 2012; Silanikove et al., 2013b). The inflammatory response in late lactation exhibits a balance response between the leukocytes composing SCC and includes elements of the acquired immune system (Leitner et al., 2012; Silanikove et al., 2013b). Thus, low milk secretion associated with balanced activation of the innate and acquired immune system allow the involution to proceed more rapidly and effectively upon induction of drying-off, to fight more effectively against existing and new infection and to clear more effectively apoptotic cells. Goats have an advantage over sheep and cows in eradicating existing infection and resisting acquiring new bacterial infection during the dry period, which is consistent with the relatively lower levels of goats which remained infected in the beginning of the new lactation (Leitner et al., 2007).

4. What can be done to improve milk quality of goats for cheese production?

It can be envisaged that mastitis, in particular SCM, will remain a major problem that will affect the quality of goat's milk for cheese production in coming years. However, the fact that in some herds from different parts of the world, SCM rate is maintained in the range of 5–20% of the goats in a given herd suggest that by applying stringent sanitizing

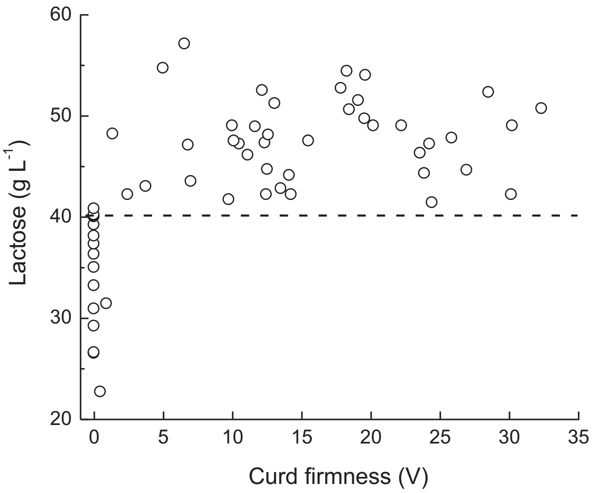

Fig. 2. Interrelationship between lactose concentration and milk clotting in goats.

measures it would be possible to improve the milk quality and thus milk yield in most herds. If dairies will apply a pricing system according to milk quality for curdling, it could motivate farmers to make the necessary changes in herd management.

The grading scheme represented in the present review can serve as a starting point for grading goat milk according to its industrial value. However, as discussed above, we are aware that bulk tank SCC level is not the ultimate criterion for grading milk according to its quality for cheese making. The level of nitrite, lactose (Fig. 2), lactate, malate and citrate in milk can be determined accurately and fast (within 2 h) and provide valuable information about curd firmness produced from the milk (Silanikove et al., 2014b). The changes in the concentration of these metabolites are consistent with sparing glucose utilization for lactose secretion for supporting the enhanced need of the immune system for energy. The level of immune responding metabolites, such as haptoglobin and serum amyloid can be determined in the bulk milk tank and provide information about it quality for producing dairy products (Akerstedt et al., 2009). These parameters have the potential for being used as much more effective tool for predicting the quality of bulk milk on the tank level for cheese production. For instance, we recently found that nitrite concentration in bacterial free gland of goat (Silanikove et al., 2014a) is 380 nM and in gland infected by CNS it go up to 860 nM. Simple calculation indicate that in herd with 20% infection with CNS, nitrite concentration in the tank level will rise to 404 nM and in herd with 40% infection nitrite concentration will increase to 476 nM. The sensitivity of the method applied for nitrite determination is ±4 nM; thus, it has the potential to detect the above-calculated changes. Applying additional parameters from the list described above may increase the sensitivity of detection milk quality for cheese yield even further. Thus, outcome of recent research may enable devising a better scheme to grade milk for industrial usage; though, obviously more research is needed in this line.

Conflict of interest

None.

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