Title: Effect of subclinical mastitis on the yield and cheese-making properties of ewe's milk

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HIGHLIGHTS

Mastitis causes a quick loss of milk yield and modification of milk composition

Compensatory production from uninfected half was quantified in unilateral mastitis

Consequences of mastitis gets worse as lactation advanced

Mastitis decreases lactose and casein:protein ratio, and increases proteolysis

Mastitis increases moisture content and losses of fat and protein in cheeses

ABSTRACT

This review covers an update of scientific knowledge about productive and technological consequences of subclinical mastitis in sheep milk. The literature reports individual milk yield losses of 2.6 to 43.1 %, being modulated by several factors as infection severity, production level, causal agents, and unilateral or bilateral IMI (1 or 2 infected glands, respectively). A

compensatory increase of milk production from the uninfected gland when only one half was infected has been quantified in 6.6%, compared with healthy halves of control sheep. This compensatory adaptation highlights the risk of underestimating subclinical mastitis in sheep. The mammary gland response is quick and milk yield losses in absolute terms remained constant within the following weeks, both when infection appear during lactation and when it is present from lambing.

With respect to the changes on main components in milk due to subclinical mastitis it has been clearly established a decrease in the concentration of lactose and an increase of that of whey proteins. The role played by lactose as an osmotic regulator result in a more accentuated decrease of its concentration in milk. This is why lactose is considered at present as a reliable potential indicator of subclinical mastitis. Whey proteins increase as a result of the increase of the blood-milk barrier permeability and the increased proteolysis of caseins. However, the content in milk of fat and casein are modified depending on the magnitude of milk yield reduction, being affected by a concentration or dilution effect. In any case, the ratio casein to protein (parameter independent of the milk volume) decreases as a result of infection.

The impairment of physical and chemical characteristics due to decreased udder health status is the responsible of the negative effect of increased SCC on the coagulation properties of milk, the curd yield and the quality of cheese. Low ratio of casein to protein in high bulk tank SCC milk enhances the extension of the rennet coagulation time (RCT) and curd firming time (k20) because there are more serum proteins and the stability of casein micelles are reduced as a result of hydrolysis. Those changes in turn led to poor syneresis, lower cheese yield, increased moisture content and lower fat and protein content in cheese.

Finally, there is a favourable relationship between lactose and milk technological properties because the decrease of this component in case of mastitis is associated with an increase of milk pH. Thus, the three parameters, SCC, pH, and lactose affect, contemporarily

and independently, milk quality and coagulation properties, and this is why have been highlighted as potential indicators traits for improving cheese-making ability of sheep milk.

Key words: subclinical mastitis, ewe milk yield, ewe milk composition, somatic cell count, proteolysis, milk cheese-making quality.

1. Introduction

The income of dairy sheep breeders comes mostly from the use of this milk kind in cheese-making; therefore both the decrease of milk yield and decline in its cheese-making quality can cause high economic losses to the farmers. Mastitis decreases milk yield and modifies its composition as a result of the damage caused in the secretory tissue of the mammary gland (Burriel, 1997; Burriel and Wagstaff, 1998).

Previous studies on dairy sheep reported that subclinical intramammary infection (IMI) by coagulase-negative staphylococci (CNS) is the single major factor affecting flock milk yield (Gonzalo et al., 2002; Leitner et al., 2008; Giadinis et al., 2012). In contrast to clinical mastitis, subclinical one is imperceptible. Frequently the glands are not treated at drying-off and the prevalence of IMI is higher during the subsequent lactation (Gonzalo et al., 2004).

The literature shows that milk yield loss in ewes with subclinical mastitis depends on infection severity, type of bacteria, time being infected, among other variation factors (Gonzalo et al., 2002; Martí-De Olives et al., 2013). On the other hand, composition and coagulation properties, which are appropriate parameters to assess the suitability of milk for cheese production, are impaired due to subclinical IMI (Leitner et al., 2004; Martí-De Olives et al. 2011; Pazzola et al., 2018). Furthermore, infected cheese milk is associated with higher losses of fat and protein in the whey during the coagulation process, and with soft and elastic cheese structure (Rovai et al., 2015).

At present, milk pricing according to quality is becoming more widespread and common for sheep milk. In this context, achievement of a certain level of quality is of interest for the

cheese making industry and for farmers, who can increase their income, not only by limiting milk yield losses, but also by improving the quality of milk.

2. Modifications in the mammary gland secretory function

The mammary inflammatory process causes an impairment of the glandular tissue. Histopathological studies in sheep showed that experimental infection by inoculation of CNS bacteria implies involution of a significant proportion of secretory cells, increase of mammary gland connective tissue and fibrous tissue formation around the affected alveoli (Burriel, 1997). In addition, biosynthesis involves a strong energy consumption that in case of mastitis competes with phagocytic cells for the glucose utilization.

On the other hand, during an inflammatory reaction the blood flow toward infection increases, resulting in increased pressure that elevates the permeability of mammary tissue allowing a higher intercellular transfer of blood substances to milk (Dhondt et al., 1977). The first consequence of that is the increase of certain blood component's concentration in milk like as serum albumin, some lipoproteins, proteases, leucocytes and Na+ and Cl- ions, which diffuse throughout the secretory cell tight junctions (Munro et al., 1984). The increase of Somatic Cells Count (SCC) in milk is the main marker for the detection and diagnosis of mastitis. Therefore, authors have considered an increase in SCC as a marker of mastitis and its level as an indicator of infection severity which can affect milk yield and quality differently (Gonzalo et al. 2002).

Besides, findings of latest years highlight the role of the plasminogen-plasmin system in reduction of milk yield and quality during IMI through plasmin-derived casein degradation products. It has been reported (Silanikove et al., 2006) that downregulation of milk secretion was associated to a slight increase of plasmin activity (10 to 40 % of the enzyme activity). This regulation entails the response induced by the fragment β-CN f1-28 derived from plasmin activity on β-CN, concerning specifically fluid secretion without affecting main

components level and integrity of the tight junction; it reduces the output of lactose and other osmotic components from the alveoli to the gland lumen. Thus reduction in milk yield due to bacterial infection would always be associated with reduced milk quality, because of the pivotal role of plasmin in these processes (Leitner et al., 2011). In addition, plasmin activity is associated with CN breakdown which decreases CN and impairs coagulation time and curd quality (Merin et al., 2008). It definitely seems that the acute reductions in milk yield and milk quality are coordinated in response to subclinical infection. According to Leitner et al., (2011) this response is more acute in sheep in comparison with cows and goats.

3. Milk yield losses and variation factors

The losses of milk yield as a consequence of a subclinical mastitis in dairy sheep are modulated by several factors as infection severity, production level, causal agents, and unilateral or bilateral IMI (1 or 2 infected glands, respectively). The literature reports a wide range of individual milk yield losses, from 2.6 to 43.1 %, depending on the above factors and according to the experimental model utilized (Table 1).

(HERE TABLE 1).

3.1. Infection severity and causative microorganisms

The most prevalent etiological group which causes subclinical mastitis in sheep is represented by staphylococci and particularly by the CNS, which induce a very variable cell response. The CNS have been historically considered as minor pathogenic microorganisms; however the study of Gonzalo et al. (2002) highlights the higher pathogenicity of a novobiocin-sensitive (NSCNS) group of CNS strains (*S. simulans, S. epidermidis, S. haemolyticus, S. caprae, S. chromogenes, S. aureus, S. hominis, S. capitis, S. auricularis, S. hyicus*) in comparison with others, since the NSCNS group induce higher SCC and production losses than minor pathogens. The ewes that were uninfected and infected by minor pathogens had the lowest SCC and yield decreases (2.6%), whereas those infected by major

pathogens had high SCC and milk yield losses that ranged between 8.8 and 10.1% according to the uni- or bilateral character of the infection, respectively. On the other hand, ewes infected by NSCNS exhibited SCC values similar to those of infections by major pathogens. The milk yield losses were between those caused by minor and major pathogens (4-5%).

3.2. Experimental Models Used to Investigate the Effects of IMI

Milk losses by IMI have been quantified by different approaches. The conventional whole-udder approach under field conditions requires a data set of numerous samples to account for the large experimental variability (Gonzalo et al., 1994, 2002). This is due to the significant individual variations between animals and to the effects of other factors, such as farm management, environmental conditions, animal husbandry, age, number and stage of lactation, production level, etc. In general, a great number of variation factors are neutralized when the units of comparison are the two glands of the same animal. The half-udder model, in which a single gland serves as the experimental unit and the contralateral gland as a control, has been extensively used to study the effect of IMI on milk yield and milk quality (Leitner et al., 2004, 2008; González-Rodríguez et al., 1995; Martí-De Olives et al., 2013). This experimental model enables the quantification of the negative effects of IMI on milk yield and quality with high statistical reliability, even for relatively small data sets of 20–40 animals.

Recently, milk losses have been quantified using both half-udder and whole-udder approaches on the same animals from an experimental flock (Martí-De olives et al., 2013). This model was reinforced with the inclusion of a pre-infection period to correct the values of the post-infection period, so that improving its precision. The individual milk loss as a result of unilateral subclinical IMI acquired during lactation was quantified by 15% in the following 7 weeks. Losses were found to be proportional to production level of animals. One of the

most important findings of this research was the quantification of a compensatory increase of milk production from the uninfected gland when only one half was infected with CNS. Under this condition, the loss in yield for the whole animal level was significantly reduced because 6.6% more milk was produced by the uninfected half, compared with healthy halves of control ewes. This compensatory adaptation, quantified by the first time in sheep, highlights the risk of underestimating subclinical mastitis in sheep. At the same time, it shows that reduction in milk yield is proportionally more important when both glands are infected than when only one. This compensatory effect (the increase from the healthy gland to compensate for the infected gland) was also described but not quantified in goats (Knight and Peaker, 1991), and was estimated to be 13% in cows (Woolford, 1985).

3.3. Evolution of milk yield loss during the infection process

Fluctuations of milk yield losses in the weeks following bacterial infection were studied in sheep (Martí-De Olives et al., 2013). It was demonstrated that the effect of IMI is fairly quick, milk yield being dramatically decreased in the infected gland and increased in the contralateral gland in the week in which infection occurred (Figure 1). These findings indicate a quick response of mammary secretor tissue to subclinical IMI, which affected milk production both in the infected glands and in their collateral uninfected ones. When infection appeared during lactation, milk yield differences in absolute terms remained constant within the 7 studied weeks. The same result was displayed within the complete lactation when infection was present from lambing. In this latest case, whilst there were not variations on the absolute magnitude of milk yield losses along the lactation period, the relative milk losses of infected glands measured with respect to that of healthy ones increased as lactation advanced, because of the typical declining trend of lactation curve (Figure 2). From this finding, it can be deduced that the consequences for milk yield of IMI occurred at the beginning of lactation could get worse as lactation advanced, as long as the glands remains infected.

(HERE FIGURES 1 AND 2)

4. Changes in milk associated with subclinical mastitis

4.1. Global changes

The changes on main components in milk due to subclinical mastitis are summarized in Table 2. In summary, it has been clearly established an increase of the concentration of whey proteins (Diaz et al., 1996; Burriel and Wagstaff, 1998; Leitner et al. 2004; Martí-De Olives et al., 2013) and a decrease in the level of lactose (Diaz et al., 1996; Burriel and Wagstaff, 1998; Bianchi et al., 2004; Leitner et al. 2003, 2004, 2011; Martí-De Olives et al., 2013; Rovai et al., 2015; Caballero et al., 2015). The decrease of lactose in milk as a result of IMI is assigned to its replacement by other osmotically active components, mainly chloride (Albenzio et al., 2004). Others also attribute the decrease of lactose to the reduction of glucose for its synthesis, as a result of the reduced blood flow to the udder in case of IMI (Vivar-Quintana et al., 2006). It is worth mentioning that lactose is an osmotic regulator of milk at cell level, so that a reduction in lactose content in milk implies a decrease of milk volume (Munro et al., 1984; Burriel, 1997). Indeed, lactose is considered as a potential indicator of subclinical mastitis (Paschino et al., 2019). However, the content in milk of other main synthesized by mammary gland components like fat and casein are modified depending on the magnitude of milk yield reduction, being affected by a concentration or dilution effect (Schultz, 1977; Burriel, 1997). In any case, when the ratio casein to protein (parameter independent of the milk volume) was established, it was confirmed that it decreased as a result of infection (Bianchi et al., 2004¸ Leitner et al., 2011; Martí-De Olives et al., 2013; Rovai et al., 2015) or elevated SCC (Pellegrini 1997; Pirisi et al., 1999). The increase of whey proteins in milk as a result of the increase of the blood-milk barrier permeability is likely the responsible for the reduction in the ratio casein to protein, because casein content does not

vary the most of cases (Díaz et al., 1996; Bianchi et al., 2004; Leitner et al., 2011; Martí-De Olives et al., 2013; Rovai et al., 2015).

Here TABLE 2

4.2. Milk proteolysis

Controlled proteolysis is an essential process during cheese-making, but it is responsible of physical and organoleptic impairments when it is spontaneous. Proteolysis occurs even into the mammary gland (Verdi and Barbano, 1991), and during conservation of milk under low temperatures (Le Roux et al., 1995). An elevated proteolysis of caseins in milk can cause anomalous rennet clotting behaviour, resulting in softer gels and low cheese yields (Klei et al., 1998).

Several studies have documented that IMI in sheep milk is associated with high proteolysis activity, plasmin being the major enzyme involved in sheep milk proteolytic phenomena related to udder inflammation (Bianchi et al., 2004; Leitner et al., 2004). Plasmin is a serine protease, whose activity into the blood is associated to the coagulation process, and it is responsible of solubilisation of fibrin coagulates (fibrinolysis). In milk, most of plasmin concentration is present under its inactive proenzyme, the plasminogen (Grufferty and Fox, 1988), whose conversion to plasmin is activated by proteolysis and regulated by activators and inhibitors (Precetti et al., 1997). There is evidence that plasmin activity in milk also increases with the advancement of lactation (Politis et al., 1989; Bastian and Brown, 1996) but it has been demonstrated that in sheep milk the role of IMI in this proteolytic activity is more important than that played by the involution processes acting on the mammary gland at the end of lactation (Albenzio et al., 2004, 2009). Thus in sheep, plasmin activity in infected halves was between 32,5% and 73,7% higher than in uninfected ones (Casoli et al., 1999; Bianchi et al., 2004; Leitner et al., 2004). When milk samples with different somatic cells was compared, plasmin activity was differentiated. According to Albenzio et al. (2011) plasmin

activity raises about 30% from samples with < 300,000 cells mL^{-1} to samples with up to 1,000,000 cells mL^{-1} and about 43% to samples with > 1,000,000 cells mL^{-1}. However, Caballero-Villalobos et al. (2018) found similar values of plasmin activity despite variations in SCC. Nevertheless, they also showed that the levels of plasmin in Manchega milk were affected by the sanitary conditions of the udder in the previous lactation, regardless of the level of SCC.

According to Bianchi et al. (2004), increased plasmin activity as a result of IMI can be explained by a higher transport of plasmin from blood to milk. On the other hand, Leitner et al. (2004) attribute this increase to the conversion of plasminogen to plasmin, that is induced by activators whose levels increase due to the increase in epithelial barrier permeability and to their synthesis by polymorphonuclear neutrophiles. Among somatic cells, polymorphonuclear cells were responsible for intense proteolysis in sheep milk whereas macrophages minimally contribute little to the proteolytic activity (Albenzio et al., 2019).

According to Silanikove et al. (2006), β-CN is the preferred substrate for plasmin, and its hydrolysis results in the production of γ-caseins and proteose-peptones (p-p); αS1-casein and αS2-casein are also susceptible to proteolysis by plasmin, and λ-caseins are products of hydrolysis of αS1-casein. However, κ-casein is resistant to proteolysis by plasmin. This way the p-p fraction and the relative proportion of casein fractions, including γ-CN, were suggested to be valid estimation predictors of endogenous proteolysis in milk with elevated SCC in cow (Le Roux et al., 1995) and sheep milk (Martí-De Olives et al., 2011). In sheep bulk tank milk the changes are significant from 500,000 cells mL^{-1} (Martí-De Olives et al., 2015).

The increased proteolysis in high SCC milk in sheep decreased mostly β-caseins; susceptibility in high SCC was in the order β- > αs2- > αs1- >> κ-CN (Pinto et al., 2013). Several studies indicated that the increased proteolysis in high SCC milk decreased the sheep

β-caseins, but did not diminish α- or κ-caseins in individual and half-udder milk (Bianchi et al. 2004; Martí-de Olives et al. 2011) and bulk tank milk (Revilla et al. 2009; Martí-de Olives et al. 2015).

In addition to plasmin, other endogenous proteolytic enzymes from somatic cells, represented mainly by elastase and cathepsins, can impair the coagulation behaviour of milk with high SCC (Albenzio et al., 2009). According to Albenzio et al. (2019), elastase is a protease associated with polymorphonuclear cells and its activity is significantly increased when these cells are massively recruited into milk during infection. Among the cathepsins, cathepsin D is identified in milk and acid whey, and plays a role in the impairment of renneting properties of sheep milk. Bovine caseins are reported to be degraded by leukocyte proteinases in the following order: α > β >> κ-CN (Considine et al., 2004). In sheep milk, Albenzio et al. (2009) indicated that elastase and cathepsin D affected primary α-casein.

5. Impact on cheese-making aptitude of milk

The first step in producing cheese is curd formation, the behaviour during curling and draining, and the curd yield define the cheese-making potential of sheep milk. In general, poor coagulation properties increase curd yield loss (Leitner et al., 2004, 2008). Several studies have documented that sheep milk with high SCC is associated with impairment of coagulation properties (Duranti and Casoli, 1991; Pirisi et al. 1996; Pellegrini et al. 1997; Bianchi et al. 2004; Revilla et al. 2009; Leitner et al., 2011; Martí-De Olives et al., 2015; Pazzola et al., 2018; Paschino et al., 2019). Globally, rennet coagulation time (RCT) and curd firming time (k20) are reported to be significantly increased with increased SCC in milk, while curd firmness after 30 min (a30), 45 min (a45) and 60 min (a60) are not significantly modified. Frequently there is a certain proportion of samples with high SCC which do not react to rennet (Bianchi et al., 2004). In a study of Manchega and Lacaune dairy sheep breeds, using the half-udder model, the rennet-clotting time of milk from infected glands with higher

SCC was twice longer than milk from the contra-lateral uninfected glands; in this case curd firmness was lower in milk from infected glands and 25–30% of them did not coagulate (Rovai et al., 2015). After blending milk from uninfected and infected glands, the study showed that a high proportion of milk from infected glands influenced uninfected milk, being responsible for worse coagulation properties and higher losses of fat and protein in the whey. Moreover, cheese structure was softer and more elastic, because of lower whey draining. In sheep bulk tank milk the changes in coagulation properties become significant from 1,000,000 cells mL^{-1} (Pirisi et al. 1999; Albenzio et al. 2004; Martí-De Olives et al., 2015).

The negative effect of increased SCC on the coagulation properties of milk and curd yield is actually a consequence of the impairment of physical and chemical characteristics due to decreased udder health status. It has been also demonstrated that the changes in milk composition associated with a high bulk milk cell count can affect the quality of cheese (Auldist et al. 1996). In sheep milk, the physicochemical analysis of the cheeses revealed that the somatic cell count level of milk has a significant influence on the amount of protein, fat, dry extract, and fatty acids (Hernández-Ramos et al., 2019). During aggregation of caseins in the process of curding, 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 (Silanikove et al., 2014). Although casein content does not change in sheep milk with high SCC or from infected ewes, the casein to protein ratio decreases as a result of an influx of serum proteins into the milk through the ruptured mammary epithelia and the breakdown of intact casein by endogenous enzymes. Low ratio of casein to protein with increased proteolysis in high bulk tank SCC milk enhanced the extension of the RCT and k20 because there are more serum proteins and the stability of casein micelles are reduced as a result of hydrolysis (Martí-De Olives et al., 2015).

Furthermore, in ewes with a previous udder infection, plasmin activity had a negative impact on rennet coagulation, probably due to casein breakdown (Caballero-Villalobos et al., 2018).

Those changes in turn led to poor syneresis, lower cheese yield, increased moisture content and lower fat and protein content in cheese (Albenzio et al., 2005; Revilla et al., 2009). Accordingly, Auldist et al. (1996) and Klei et al. (1998) report that casein to protein ratio is the parameter related to protein fraction of milk that best explains the cheese yield and protein recovery variations due to SCC. Besides that, milk lactose content appeared to be one of the components most linked to gelation, curd firming time and water retained in the curd because of its relationship with udder health (Vacca et al., 2019). According to these authors there is a favourable relationship between lactose and milk technological properties because the decrease of this milk component is associated with an increase of milk pH. Since the decrease of sheep lactose content is associated to a linear increase in SCC, milk acidity is reduced in milk with high SCC, affecting the whole coagulation process. In that regard, Pazzola et al. (2018) investigated the effect of somatic cell count, lactose, and pH as markers of udder infection on sheep milk composition, coagulation properties and curd firming parameters. Results reported that SCC, pH, and lactose affect, contemporarily and independently, milk quality and coagulation properties. These parameters, measured by infrared spectroscopy during milk collection prediction, could be used as potential indicators traits for improving cheese-making ability of sheep milk.

Actually, one of the most important factors in curd structure formation is the pH. At low pH, calcium is progressively dissociated from the casein micelle, and neutralises the negative charges of the different casein fractions, favouring extensive aggregation and fusion between the micelles which tend to form a casein network in which the other components of coagulum are entrapped (Park, 2007). Coagulation parameters are affected by pH and calcium content. The increased pH of milk with SCC (Raynald- Ljutovac et al., 2007; Martí-De Olives et al.,

2015), could negatively affect both the first phase of rennet coagulation and the aggregation casein micelle due to a lower Ca^{+2} activity that is also frequent in mastitis milk (Caballero et al., 2015). In fact, mastitis is also responsible of a greater transfer of Ca^{+2} from milk to blood, which contributes to a longer RCT of sheep milk (Balcones et al., 1996). With respect to this important effect of pH, Caballero et al., (2015) found that standardising milk pH at 6.5 prior to rennet addition can improve the coagulation properties of milk with high SCC.

6. SCC as milk quality parameter for cheese-making

Bulk tank milk parameters have been the target of different legal limits or payment-by-quality schemes proposed by different regions, with obvious repercussion on milk marketing (Directive 94/71/EEC; European Union, 1994). However, BTSCC (bulk tank SCC) values in sheep and goat milk used for dairy products sold in the European Union has yet to be regulated. The bulk milk is a mixture of all the animals milked at a certain moment; therefore the quantity and quality contribution of each individual animal is minimal. However, the influence of infected animals or glands on SCC of tank milk is high due to the low volume of milk into the tank.

Although there is a clear trend showing the inverse relationship between high SCC and sheep milk yield or milk quality, using only the SCC level for such predictions is not sufficient owing to variation factors other than IMI, like the different dairy breeds and management systems, bacteria species involved, time in lactation and different end products (Bianchi et al., 2004; Raynald-Ljutovac et al., 2007). Because of this, local calibration standards for BTSCC determination are required (Leitner et al., 2016).

Three grades or categories of sanitary quality of milk have been proposed relating to the BTSCC and IMI prevalence in Churra breed sheep (Ariznabarreta, 1999): grade A or good (BTSCC < 500,000 and < 30% of infected ewes into the herd), grade B or average (BTSCC between 500,000 and 1,000, 000 and 30% < infected ewes > 40%) and grade C or bad

(BTSCC > 1,000,000 and > 45% of infected ewes). Based on these categories, Arias et al. (2015) estimated the minimal milk production losses 12.24% and 8.71% in herds with BTSCC > 1,500,000 cells/ml and BTSCC < 500,000 cells/ml, respectively. Also, losses of milk and curd yield at the herd level in relation to IMI have been evaluated in herds of Assaf sheep (Leitner et al., 2008). Recommendations of these authors on how goat and sheep milk should be graded for industrial use are presented in Table 3.

(HERE TABLE 3)

7. Conclusions

The importance of subclinical mastitis on sheep milk yield and quality is unquestionable, so that the prevention and control mechanisms of this illness are very important to minimize the economic losses that can cause. It is possible to underestimate the effects of subclinical mastitis on milk production under unilateral IMI conditions, due to the tendency of healthy gland to compensate the milk yield lost in infected gland.

Technological quality is clearly affected by subclinical mastitis. The use of additional parameters in the quality milk price systems, for predicting the cheese-making milk aptitude in relation to IMI, could be implemented. For exemple the casein to protein ratio and the content of lactose since are more clearly related to mastitis. In most of the studies, the pathogen responsible for sheep mastitis was not identified. Nevertheless, the different effect of subclinical IMI on many milk components could be explained by the different impact of the causative microbial agents on the mammary gland immune response. Actually, studies on the impact of mastitis on cheeses are scarce and the impact of the different causative agents should be tested.

In relation with BTSCC, it seems that it is still possible to use SCC as a criterion for sheep milk quality. Nevertheless, research is still needed to study sheep milk quality parameters in relation to SCC at bulk tank level, especially in terms of cheese-making ability.

Declarations of interest:

None.

Acknowledgments

This review is based on knowledge gained during work financially supported by means of a research fellowship from the regional government of Valencia ("Generalitat Valenciana").

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FIGURES

Figure 1. Productive potential of milk (PPM) of infected A glands (○), B contralateral to A glands (□), C glands of healthy control ewes (▲) and D glands of healthy control ewes (♦), before the onset of infection (IW < 0) and after the onset of infection (IW ≥ 0) (Martí-De Olives et al., 2013).

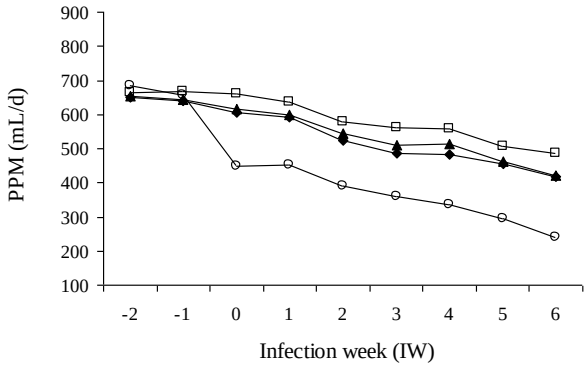
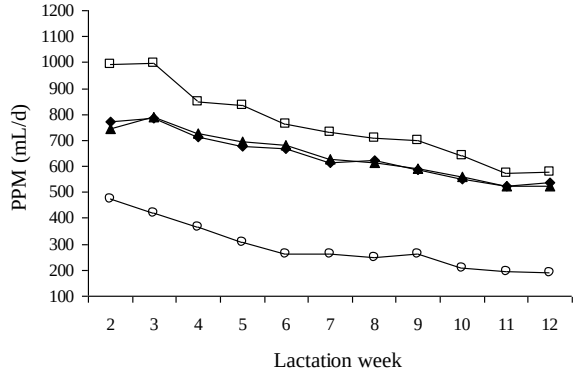


Figure 2. Productive potential of milk (PPM) throughout lactation of infected A glands (○), B contralateral to A glands (□), C glands of healthy control ewes (▲) and D glands of healthy control ewes (♦), during lactation period (Martí-De Olives et al., 2013).



TABLES

Table 1. Individual milk yield loss as affected by subclinical IMI according to causal agent, SCC and model approach. Data from several authors.

Milk yield loss (%)		Causal agent	SCC (x 10^3 cells/ml)		Model approach	Breed	Reference
Unilat. Inf. [1]	Bilat. Inf. [1]		Unilat. Inf.	Bilat. Inf.			
-	37	*S.simulans*	-	-	Whole udder	Meat breeds	Fthenakis and Jones (1990)
9.9	31.6		>1000	>1000	Whole udder	Altamura, Leccese, Comisana, Sarda	Dario et al. (1996)
15	-	CNS, *Str. Bovis*	3500		Half and whole udder	Manchega	Peris et al. (1996)
-	43.1	*S.epidermidis*		>1000	Whole udder	Karagouniko	Saratsis et al. (1999)
2.6	-	Minor Pathogens	120				
5.1	3.6	NSCNS[2]	597	1547	Whole udder	Churra	Gonzalo et al. (2002)
8.8	10.1	Major Pathogens	1317	2351			
2.8	37.2	CNS, *S. aureus, Streptococci Pseudomona, Corynebacterium*	141	2089	Whole udder	Israeli-Assaf Israeli-Awassi	Leitner et al. (2003)
-	52.6*	CNS	2358		Half udder	Israeli-Assaf	Leitner et al. (2004)
15	-	CNS	1622		Half and whole udder	Manchega	Martí-De olives et al. (2013)

[1]Unilat. Inf.: unilateral infection; Bilat. Inf.: bilateral infection;
[2]NSCNS: novobiocine-sensitive coagulase-negative staphylococci.
*Loss at gland level: difference between infected glands and uninfected ones.

TABLE 2

Table 2. Composition (%) of normal (N) and mastitic (M) sheep milk according to several authors.

Lactose		Fat		P		CN		CN:P		Whey protein		SCC[1]	Breed	Reference
N	M	N	M	N	M	N	M	N	M	N	M	M		
4.99	4.76 ↓	9.07	8.90 ↓	5.43	5.65 ↑	4.30	4.29 =	76.63	74.77↓	0.86	1.02 ↑	2506	Manchega	Díaz et al. (1996)

4.65	4.51 ↓	7.06	6.32 ↓	5.47	5.95 ↑	4.18	4.45 =	76.73	74.77↓	-	-	>1000	Sarda	Bianchi et al. (2004)
5.30	4.72 ↓	4.68	5.29 ↑	5.13	5.50 ↑	-	-	-	-	-	-	1288	Assaf	Leitner et al. (2003)
4.47	3.35 ↓	6.49	6.17 ↓	5.85	5.35 ↓	4.59	4.05 ↓	-	-	1.19	1.28 ↑	2358	Assaf	Leitner et al. (2004)
4.79	4.05 ↓	7.27	6.87 ↓	4.77	5.01 =	2.31	2.33 =	74.16	68.26↓	-	-	7211	Assaf	Leitner et al. (2011)
4.92	4.56 ↓	8.52	8.65 ↑	5.50	5.81 ↑	4.50	4.69 =	80.29	78.58↓	0.84	0.97 ↑	1622	Manchega	Martí-De Olives et al. (2013)
4.71	4.19 ↓	8.06	8.18 =	6.9	6.75 ↓	5.52	5.39 =	80.00	79.90↓	-	-	1995	Manchega	Rovai et al. (2015)
4.55	4.41 ↓	8.37	8.30 ↓	6.25	6.41 =	-	-	-	-	-	-	1705	Manchega	Caballero et al. (2015)
4.87	4.66 ↓	6.28	6.54 ↑	5.23	5.48 ↑	4.08	4.26 ↑	-	-	-	-	3235	Sarda	Paschino et al. (2019)

Arrows indicate the effect on milk: an increase (↑), a decrease (↓) or not statistically significant effect (=).
[1] SCC (x 10^3 cells/mL)

Table 3. Estimated losses in milk and curd in sheep due to herd infection level (Prevalences).

Herd infection level	BTSCC (cells/ml)	Milk loss (%)	Curd loss (%)	Reference
Grade A: 0–25%	450,000–800,000	0–4.1	0–5.2	Leitner et al. (2008)
Grade B: 25–50%	800,000–1,400,000	4.1–8.2	5.2–10.4	
Grade C: 50–75%	1,400,000–2,000,000	8.2–12.2	10.4–15.5	
Grade A: < 30%	< 500,000	0–8.7	-	Arias et al. (2015)
Grade C: > 50%	> 1,500,000	8.7–12.2	-	