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16 **ABSTRACT**

17 Mastitis is an inflammation of the udder, mainly caused by bacteria, and leads to economic
18 loss, due to discarded milk, reduced milk production, reduced milk quality, and increased
19 health costs in both dairy sheep and cattle. Selecting for increased genetic resistance to
20 mastitis can be done directly or indirectly, with the indirect selection corresponding to a
21 prediction of the bacteriological status of the udder based on traits related to the infection.
22 The most frequently used indirect method is currently milk somatic cell count (SCC) or
23 somatic cell score (SCS). This review reports the state of the art relating to the genetic basis
24 of mastitis resistance in sheep, and explores the opportunities to use SCC as selection
25 criterion in a breeding programme to improve resistance to mastitis in sheep, discussing the
26 actual situation and prospects for improvement. It has been stressed, in particular, that
27 although it is unlikely that selection for mastitis resistance by the farmers on their own will be
28 successful, there is good prospect for genetic improvement if reliable pedigree and
29 performance recording is implemented across flocks, combined with breeding value
30 estimation. To achieve this, a strong and well-structured organization to implement and
31 support the program is essential.

32

33 **Key words**: mastitis, genetic selection, somatic cell count, sheep

34

35 **1. Introduction**

36 The Mediterranean Basin countries host 60% of the total world sheep and goat milk
37 production. The dairy sheep and goat industry is usually based on local breeds, which are
38 very well adapted to the production systems and environments. Milk production is the
39 principal trait affecting the profitability of these industries, and therefore for long time the
40 breeding programmes have considered milk production as the major selection criterion.

However, due to the EU agricultural policy and consumer demands, increased attention has been focused on traits related to the reduction of production costs, food safety and health (e.g. resistance to intramammary infections, internal parasites, scrapie, etc.). Mastitis, in particular, is one of the main infectious diseases in dairy sheep and goats as well as in dairy cattle – with respect to dairy industry and public concern, economic impact, zoonotic potential and animal welfare (Davies et al., 2009).

This review reports the state of the art relating to the genetic basis of mastitis resistance in sheep, and explores the opportunities to use somatic cell count (SCC) as a selection criterion in a breeding programme to improve the resistance to mastitis in sheep, discussing the actual situation and prospects for improvement.

2. Mastitis and mastitis-causing pathogens

Mastitis is an inflammation of the udder and it leads to economic loss, mainly due to discarded milk, reduced milk production and quality and increased health costs (Miller et al., 1993; Allore and Erb, 1998; Leitner et al., 2003). Rupp and Foucras (2010) reported that the total annual milk production losses due to mastitis in small dairy ruminants can be estimated to be in the region of €60 million/annum.

Mastitis can be classified as subclinical or clinical. Mastitis is subclinical when no visible changes occur in the appearance of both the milk and udder, but milk production decreases, bacteria are present in milk and the milk composition is altered (Harmon, 1994). On the other hand, mastitis is clinical when symptoms such as fever, abnormal texture and discoloration of the milk, increased temperature or pain of the quarter or udder half, and a change in milk properties occur. Generally, the incidence of clinical mastitis in cattle varies between 20 and 40% per cow/year (Heringstad et al., 2000). In small ruminants, the annual incidence of

clinical mastitis is generally lower than 5% (Bergonier and Berthelot, 2003; Contreras et al., 2007), whereas the incidence of subclinical mastitis in these species has been estimated at 5-30% per lactation or even higher (Bergonier and Berthelot, 2003; Contreras et al., 2003).

Mastitis-causing pathogens include bacteria and non-bacterial pathogens, like mycoplasmas, fungi, or viruses (Bergonier and Berthelot, 2003). Among viruses, the Maedi-Visna virus is one of the main causes in sheep, having being associated to mastitis, as well as chronic inflammatory lesions in the lungs, joints, and brain (Radostits et al., 2007). However, given that the occurrence of non-bacterial pathogens is far less frequent, they will not be further considered in this review.

The bacterial pathogens responsible for infection of the mammary gland (in particular coliform bacteria, staphylococci and streptococci) may be split into two main categories, according to the severity of the clinical signs, namely major and minor pathogens. Infection with major pathogens generally results in clinical illness or strong inflammatory responses and reduced milk yields, whereas minor pathogen infection is usually subclinical (White et al., 2001). Pathogens can also be categorised, depending on their aetiology, into environmental or contagious (Fox and Gay, 1993):

i) Environmental bacteria (found in the soil, faeces, and bedding), which enter the teat duct from these sources and include both Gram-positive and Gram-negative bacteria such as *Streptococcus non-agalactiae* and coliform organisms (*Escherichia coli*, *Klebsiella* sp., *Aerobacter aerogenes*, *Enterobacter* sp.);

ii) Contagious bacteria, which are transmitted from infected quarters or halves to non-infected quarters or halves during the milking process and include such Gram-positive bacteria as *Staphylococcus aureus* and *Streptococcus agalactiae*.

88 In cattle, coagulase-negative staphylococci (CNS) are considered to be minor pathogens; this,
89 however, is less clear in sheep, in which CNS are considered the most common bacterial
90 species causing both subclinical and clinical mastitis (Albizu et al., 1991; Amorena et al.,
91 1991; Marco et al., 1991). In chronic cases, Gonzalo et al. (1998) suggested dividing the CNS
92 into two groups with different pathogenicity between dairy sheep: NRCNS (novobiocin-
93 resistant CNS), which behave as minor pathogens, resulting in mild changes in SCC and milk
94 yield and similar to those commonly associated with micrococci and Corynebacteria (Ziluaga
95 et al., 1998). Also NSCNS (novobiocin-sensitive CNS), which cause more substantial
96 changes in SCC and loss in milk yield, similar to those associated with the classic major
97 pathogens (Peris et al., 1996).
98
99 **3. Selection criteria to select for mastitis resistance**
100 Mastitis resistance is a complex trait, involving both genetic and environmental factors,
101 including infection pressure. In the broadest sense, resistance could be defined as the ability
102 to avoid any infection and/or the quick recovery from an infection (Rupp and Boichard,
103 2003). It involves different components, namely avoiding entry of the pathogen into the
104 mammary gland, mounting an immune response capable of limiting its development in the
105 udder and clearing the infection, as well as controlling the pathogenic effects of the infection,
106 such as, e.g., tissue damage (Rupp and Foucras, 2010).

107 Selecting for increased genetic resistance to mastitis can be done directly or indirectly. Direct
108 selection relates to the diagnosis of the infection. The actual trait (e.g. bacteriological
109 examination of milk and/or observation of clinical cases of mastitis) is measured on the
110 animal or its relatives. Indirect selection relates to a prediction of the bacteriological status of
111 the udder, based on traits related to the infection (e.g. inflammatory parameters). In this case,

112 an indicator trait for mastitis is measured on the animal itself or its relatives (de Haas, 2003).
113 A direct bacteriological assay is the recommended method of diagnosis of mastitis
114 (González-Rodríguez and Cármenes, 1996), as it is believed to provide precise and
115 exhaustive information on infected quarters and/or halves and the pathogens involved.
116 However, it is rarely used for genetic purposes, because it is difficult to implement on a large
117 scale. It also has limitations because of the requirement of intensive labour, the time delays
118 for culture to occur, and the costs involved with bacteriology (McDougall et al., 2001).
119 Moreover, it has been shown that bacterial shedding is variable and levels may sometimes be
120 too low to be detected by conventional techniques (Rupp and Foucras, 2010). Therefore,
121 although the bacteriological examination is often considered to be the 'golden standard' for
122 routine detection and identification of mastitis pathogens, it has to be taken into account that
123 even good quality bacteriological data will have true sensitivity and specificity values
124 somewhat less than one, i.e. some cases will be missed and others will be misdiagnosed as
125 infected when they are not (Riggio et al., 2010).

126 Simple, indirect methods have been widely applied, based on the evaluation of the degree of
127 inflammation or of internal mammary lesions (De la Cruz et al., 1994). Their accuracy is
128 usually established by bacteriological analysis as a reference method. Among these methods,
129 the most frequently used to detect mastitis is SCC.
130

131 **4. Biological signification of SCC**

132 Somatic cells normally occur in milk of both cattle and small ruminants. Somatic cells consist
133 of many types of cells, including polymorphonuclear leukocytes (PMN), macrophages,
134 lymphocytes, eosinophils, and various epithelial cells from the mammary gland. Cells in milk
135 from a healthy udder are mainly represented by mammary gland epithelium and drain canal

136 cells. Recently, Leitner et al. (2012) showed that epithelial cells accounted for ~50% of the
137 cells in goats and cows, whereas in sheep this was ~80%. These researchers suggested that
138 sheep shed more epithelial cells into milk in comparison to cows and goats, probably because
139 these cells play an important role in the immune response. According to Walawski (1999)
140 only 8% of the cells are leukocytes and less than 1% are macrophages in cattle. However, in a
141 more recent study Leitner et al. (2012) showed that in bacterial free animals at midlactation,
142 goats had the highest number of leukocytes and PMN. Sheep, on the other hand, had the
143 lowest and cows were intermediate between sheep and goats. It has also been reported that
144 PMN are the major cell population during early inflammation and play a protective role
145 against infectious diseases in the mammary gland (Kehrli and Shuster, 1994; Persson-Waller
146 et al., 1997). Experimental intramammary infection of sheep with *Staphylococcus aureus* or
147 *Escherichia coli* has been shown to induce a significant increase in PMN within 24 h of
148 infection (Persson-Waller et al., 1997).

149 Determination of the differential cell count in milk is another useful approach to evaluate the
150 proportion of leukocytes during inflammation and thus the immune status of the mammary
151 gland. In ewe milk samples, flow cytometry was used to detect the percentage of PMN,
152 macrophages, and lymphocytes in bulk and individual milk with different concentrations of
153 somatic cells (Albenzio et al., 2009; Albenzio and Caroprese, 2011; Albenzio et al., 2011).

154 The concentration of somatic cells in milk is defined as SCC and it is expressed as thousands
155 of cells per millilitre of milk. The measure of SCC has the following properties:
156 it can be routinely recorded in most milk recording systems;
157 the heritability of SCC is higher than the heritability of the direct trait (i.e., mastitis
158 incidence);
159 it is usually an indicator of both clinical and subclinical infections.

160 What is reported thus far shows why SCC is usually considered as a good predictor of
161 mastitis occurrence (milk SCC reflects the number of neutrophils migrating from blood to the
162 mammary gland in response to infection). However, numerous factors influence the SCC
163 level of both infected and non-infected animals, such as the physiological status of the host,
164 the infection status and the pathogen. It is, therefore, difficult to interpret single measures and
165 define fixed thresholds, as distributions of the SCC of infected and non-infected animals
166 overlap considerably (Riggio et al., 2010; Rupp and Foucras, 2010). This aspect will be
167 further analysed in the next sections. From these considerations, it follows that repeated
168 measures or lactation average are usually preferred for both diagnosis and genetic purposes.

169 The distribution of SCC is positively skewed; whereas, conventional statistical methods
170 usually accommodate normally distributed data. In order to obtain a distribution which
171 closely resembles a normal distribution, the SCC is log-transformed to somatic cell score
172 (SCS). The formula commonly used is: $SCS = \log_2(SCC/100) + 3$ (Ali and Shook, 1980).
173 However other researchers have used either $\log_e$ or $\log_{10}$ logarithmic transformation (Samoré,
174 2003).

175

176 ***4.1. SCC in sheep***

177 While cattle SCC values between 250 and 300×10^3 cells/mL are reported as most satisfactory
178 discrimination thresholds between healthy and infected udders, sheep do not have a widely
179 accepted threshold. Some evidence has been provided that healthy ewes have normally higher
180 SCC than cows (Maisi et al., 1987; Fthenakis et al., 1991; González-Rodríguez et al., 1995).
181 Bufano et al. (1996) showed that a high SSC (>1 million/mL) occurs in healthy sheep and
182 goat milk, especially towards the end of lactation. While Riggio et al. (2010) reported that the

183 SCC can be high, even when ewes are not infected, suggesting that a healthy animal can
184 wrongly be diagnosed as infected based on SCC.

185 On the other hand, considering subclinical mastitis, Leitner et al. (2008) suggested that, while
186 in dairy cows subclinical mastitis is largely ignored, because the increase in SCC in infected
187 glands is modest (about 300-500×10^3 cells/mL) and the mixing with the milk from non-
188 infected quarters is sufficient in most cases to appreciably lower the effect of SCC at the cow
189 level. In sheep and goats, which have only two mammary glands, mixing of milk with high
190 SCC coming from an infected gland with a low SCC from a healthy gland might be
191 insufficient to reduce the SCC at the animal level. However, whether these high SCC are a
192 consequence of the fairly generalized lack of preventive management measures against
193 subclinical mastitis in sheep flocks or whether a higher cell discrimination threshold is
194 required for sheep milk, has not been established.

195 It is important to highlight, however, that the choice of a threshold in the cattle industry was
196 mostly driven by monetary factors. While little knowledge has been available on the
197 significance of other factors in keeping farmers motivated to improve mastitis management
198 (Valeeva et al., 2007). In sheep, some studies reported that similar payment systems (e.g.
199 reduced milk prices, if the SCC of the bulk tank milk exceeds certain thresholds) are
200 becoming common (Legarra et al., 2007; Pirisi et al., 2007). However, the current milk
201 payment system of most breeds and countries is still based only on milk yield and not on
202 SCC level. This makes it more difficult to choose a threshold to discriminate between healthy
203 and infected udders, which can be worldwide accepted. Some researchers (Fthenakis et al.,
204 1991; Jones, 1991) reported discrimination values between healthy and infected glands
205 ranging from 500 to 1600×10^3 cells/mL, while others (Bergonier et al., 1994; De la Cruz et
206 al., 1994; Pengov, 2001) reported values similar to those for cows (200 to 300×10^3 cells/mL).

González-Rodríguez et al. (1995) suggested that breed differences in SCC do exist. Considering several breeds, these researchers reported the value of 300×10^3 cells/mL as the most suitable threshold of discrimination for total SCC data. However, within each breed the most suitable threshold was 400×10^3 cell/mL for Assaf and Castellana and 200×10^3 cell/mL for the Churra sheep breeds.

Recently, it was also suggested that SCC diagnostic effectiveness (SCC ability to detect whether or not intramammary infections occur) may be assessed to a degree without having to commit to a single threshold with the use of average indices based on Receiver-Operating Characteristic (ROC) curves (Riggio et al., 2013). These researchers identified different optimal SCS thresholds, ranging from 2.81 to 3.33, depending on the trait definition (e.g. SCS for the whole sample, SCS for samples with minor pathogen infections, and SCS for samples with major pathogen infections). It was suggested that different SCC (and therefore SCS) thresholds should be used when considering mastitis caused by minor or major pathogens.

5. Genetic parameters of SCC and mastitis and correlations with other traits in sheep

5.1. Genetic parameters of SCC and mastitis in sheep

Genetic studies of SCC in dairy sheep are more recent and less frequent than in dairy cattle. Heritability estimates, based on repeatability test-day models, range from 0.04 to 0.16 for several breeds including the Churra (Baro et al., 1994; El-Saied et al., 1998; Othmane et al., 2002), the Manchega (Serrano et al., 2003), the East Friesian (Hamann et al., 2004) and the Valle del Belice sheep breeds (Riggio et al., 2007). Other studies reported similar or slightly higher heritability estimates (from 0.11 to 0.18) for the average SCS during lactation, for Chios (Mavrogenis et al., 1999), Lacaune (Barillet et al., 2001; Rupp et al., 2003a), Latxa

231 (Legarra and Ugarte, 2005) and Manech Red Faced ewes (Barillet et al., 2008). These
232 heritability estimates are comparable to those reported in literature for cattle either with test-
233 day (Carnier et al., 1997; Mrode et al., 1998) or lactation models (Rupp and Boichard, 1999).
234 Moreover, in cattle it has been shown that heritability estimates for SCS are usually higher
235 than heritability for the direct trait (i.e. mastitis incidence). Therefore, when only considering
236 the heritability, these results suggest that selection for SCS (as indicator of mastitis) has to be
237 preferred over selection for the direct trait. However, before conclusions can be drawn,
238 correlations between traits should be considered.

239 In cattle, for example, genetic correlations between SCS and the incidence of clinical mastitis
240 vary from moderate to high, with an average of approximately 0.7 (Rupp and Foucras, 2010).
241 These results, therefore, confirm that, although SCS and mastitis are not the same trait, SCS
242 can be used as a selection criterion in a breeding programme for mastitis resistance in cattle.
243 In sheep, however, no estimates of genetic correlations between SCC and clinical and
244 subclinical mastitis incidence have been reported in the literature.

245 On the other hand, when considering data on intramammary infections assessed by
246 bacteriological analyses, only few results are found in the literature. Published studies refer
247 more directly and exhaustively to udder health status. In cattle, heritabilities for
248 intramammary infections varied from 0.02 to 0.04 as reported by Weller et al. (1992).
249 Somewhat higher (0.10 to 0.20) as quoted by Detilleux et al. (1994) and Wanner et al. (1998).
250 In sheep an estimate of 0.09 for the infection status assessed by bacteriological analyses was
251 reported by Riggio et al. (2010) and Tolone et al. (2013) in the Valle del Belice breed.
252 However, it was reported that with imperfect sensitivity and, particularly, specificity, the
253 heritability of liability is likely to be substantially underestimated. In other words, there may
254 truly be more genetic variation for the liability to mastitis than the field data suggests (Riggio

et al., 2010). Tolone et al. (2013) reported a genetic correlation between SCS and the infection status, as assessed by bacteriological analyses of 0.93, suggesting that selection for low SCS could also lead to a reduced incidence of mastitis. These results, therefore, indicate that selection for reduced SCS can help to reduce mastitis incidence. In this regard, results by Rupp et al. (2009) from a first-lactation survey in dairy sheep have provided evidence that selection based on SCS estimated breeding values (EBVs) may help to improve resistance to clinical and subclinical mastitis. Low SCS line animals showed a lower incidence of clinical mastitis, a lower prevalence of mammary abscesses and subclinical intramammary infections, especially at parturition. A better ability to recover from intramammary infections contracted during lactation and a lower SCS in bacteriologically positive samples was also found. These results were also emphasized by Riggio et al. (2010), suggesting that animals with a high SCS in bacteriologically negative samples, are more prone to mastitis. Therefore, the approach of selecting animals for decreased SCS is justified and should help to reduce the prevalence of mastitis, even in the absence of knowledge about infection status of the animal. This is in agreement with what previously reported in cattle. Philipsson et al. (1995) have estimated a linear relationship between SCC and the occurrence of clinical mastitis – concluding that the selection for lower SCC was desirable and that a lower level of SCC reflects a reduced incidence of infection, rather than a reduced ability to react to it. Moreover, Rupp et al. (2000) concluded that cows with the lowest mean SCC in the first lactation had the lowest risk for clinical mastitis in the second lactation. These results, therefore, suggest that breeding goals should favour animals with the lowest observed SCC. Nevertheless, it has been stated that by decreasing the milk SCC to very low levels by selection, could impair the animal's capacity to combat intramammary infection. Some of the milk resident cells, such as macrophages, are essential in initiating the inflammatory process in response to intramammary invading pathogens. Therefore, it might be useful to monitor if this (i.e.

selection for the lowest SCC level) does not affect the ability to resist infections. A better understanding of the defence mechanisms affected or modified by such a selection could be indeed helpful, to predict indirect responses on udder health in the long term and, if necessary, to modify the selection modality and criteria accordingly. It could also be important to monitor the actual mastitis incidence in the population by, for example, collecting information on the infection status at regular intervals to ensure that selection on correlated traits still results in the desired improvement of udder health.

When deciding upon the most appropriate trait to select for, one should also take into account the sociocultural background of the farmers. Compared to the collection of information on infection status or clinical mastitis, it is easier, cheaper, and less time-consuming for farmers to collect information on SCC. This can be regularly recorded during milk recording at a low cost. In this case, therefore, farmers would likely be more willing to cooperate because of the low costs and high frequency of recording. In contrast, samples for determining the infection status have to be collected with more care, than samples for SCC. The implementation of a protocol for collecting such samples by farmers may be difficult, requiring more commitment in order to ensure sufficient quality of sample collection. It may therefore also be necessary, in this case, to have these samples collected by more qualified persons, with the obvious disadvantages of higher costs and additional time by the farmers.

It is important to highlight, however, that in most of the sheep breeds, current selection is mainly practised on a “within farm” basis and based on the performance of the ewes. In this situation, according to the considerations drawn so far, it is unlikely that selection for mastitis resistance will be successful – independent of the use of infection status or SCS. Based on the above considerations, therefore, the implementation of a well-structured breeding programme needs to be realized, in order to guarantee reliable pedigree recording and performance

304 registration. At present, only a few dairy populations worldwide, mainly located in the
305 Mediterranean region or in North America, have the required organization to allow the
306 development of a large-scale recording and genetic evaluation (Rupp and Foucras, 2010). To
307 current knowledge, the French Lacaune breed is the only small ruminant dairy breed selected
308 for increased udder health (Rupp et al., 2002) – with genetic evaluations for the lactation
309 mean SCS, run since 2002, based on a simplified recording system for SCC and implemented
310 in the same way as that for milk fat and protein content (Rupp et al., 2002).
311

312 ***5.2. Genetic correlations between SCS and other traits***

313 Although farmers select on several traits, based on own performance, milk yield is currently
314 the most important selection criterion, for which phenotypic records are collected and
315 breeding values are estimated, in most dairy sheep breeds. Barillet (1997) suggested that the
316 introduction of milk composition traits and/or functional traits (e.g. resistance to mastitis) as
317 selection objectives should be addressed only when a breeding programme has reached an
318 asymptotic annual genetic gain for milk yield. However, this ignores the correlated response
319 in other economically important traits, resulting from selection on milk production only. To
320 quantify the likely correlated responses, it is important to determine the genetic correlations
321 between different traits.

322 Unlike bovine mastitis, where the genetic antagonism between SCS and milk production
323 traits is well documented, genetic correlation estimates between milk production and mastitis
324 traits are quite inconsistent across dairy sheep studies. Published genetic correlations between
325 SCS and milk yield range from positive i.e. antagonistic, to negative (Baro et al., 1994; El-
326 Saied et al., 1998; El-Saied et al., 1999; Barillet et al., 2001; Rupp et al., 2003a; Riggio et al.,
327 2007).

328 Another interesting aspect to consider is the correlation between SCS and udder conformation
329 traits, which are favourable according to literature (Legarra and Ugarte, 2005; Sechi et al.,
330 2007). Results suggest that udders with what is perceived to be a good shape are less affected
331 by sub-clinical mastitis. Pendulous udders have been associated with an increase in SCC
332 (Casu et al., 2010; Huntley et al., 2012). Pendulous and deep, poorly attached udders are
333 difficult to milk and may cause sudden cluster falling, teat-end impacts, and subsequent
334 bacterial infections (Bergonier et al., 2003). In addition, these udders are more prone to
335 injuries (Legarra and Ugarte, 2005). However, this is a bit controversial, as Huntley et al.
336 (2012) showed that teat lesions were not significantly associated with a change in udder half
337 SCC, suggesting that teat lesions do not increase the risk of bacterial invasion of the udder.
338
339 **6. Alternative statistical modelling for SCC/SCS**
340 In using SCC as an indicator of mastitis, the dynamic nature of mastitis is often ignored in the
341 statistical analysis. It has been reported that both clinical and subclinical mastitis cause
342 deviations from a typical curve of SCC (de Haas et al., 2004). In this respect, the use of
343 individual SCC test-day records is an improvement, compared to the average of SCC records
344 collected during a lactation. However, Urioste et al. (2010) reported that the use of test-day
345 SCC can still make it difficult to identify short-duration infections, as SCC is often only
346 recorded at approximately monthly intervals. Therefore, Urioste et al. (2010) suggested
347 exploring alternative traits derived from the SCC curve (e.g. traits designed to capture SCC
348 base levels and variation along the curve, time and level of infection, and time of recovery).
349 Ideally, these alternative traits should be able to accommodate sudden and drastic changes in
350 SCC, which in turn may improve the diagnosis of mastitis and hence increase genetic
351 progress in mastitis resistance. There are, however, limitations to the use of these alternative
352 traits on commercial farms. If it is true that the shortcoming of SCC is that it is only recorded

353 monthly, making it difficult to identify short-duration infections, then these alternative traits
354 are unlikely to contain more information as they are based and designed on the same original
355 information (i.e. test-day SCC). Moreover, ewes are milked (and, therefore, SCC records
356 available) only once lambs are fully weaned, which could lead to an early misclassification of
357 healthy and infected animals. Therefore, these alternative traits can probably be explored,
358 used and better exploited on experimental farms, where the SCC records can be collected
359 more frequently.

360 In the genetic evaluation of SCS, information collected on healthy (i.e. non-infected) and
361 infected animals, is treated equally. However, several researchers suggested that, in cattle,
362 SCS in healthy and infected animals are different traits (Detilleux and Leroy, 2000; Boettcher
363 et al., 2007; Madsen et al., 2008). This was also confirmed in sheep by Riggio et al. (2010),
364 who showed that SCS in healthy and infected animals can indeed be considered as different
365 traits – with different heritabilities, and with a genetic correlation between bacteria negative
366 and bacteria positive SCS of 0.62. Whilst this genetic correlation is moderately positive, it is
367 significantly less than unity, suggesting that bacteria negative and bacteria positive SCS are
368 not the same trait. The genetic evaluation of SCS can be improved when this non-unity
369 genetic correlation is taken into account. In most countries, however, cases of mastitis are not
370 routinely recorded in a systematic manner. The lack of information on the infection status is a
371 limitation in selecting directly for mastitis resistance. It implies that when using SCS as an
372 indicator of mastitis, no distinction can be made between SCS data from infected and non-
373 infected animals.

374 When information on the infection status is not available, SCS may be regarded as a mixture
375 of observations from animals with unknown health status, i.e. with and without mastitis.
376 Mastitis infection would produce a deviation from the SCS baseline level, i.e. an observed

test-day SCS can be regarded as resulting from effects of a baseline SCS (a continuous trait) and a deviation caused by a binary process (healthy or infected status). Detilleux and Leroy (2000) have shown that a finite mixture model can account for these differences and can represent a latent structure in a set of data, whereby observations may belong to one of several distributions – possibly differing in mean, variance, and even the type of distribution (McLachlan and Peel, 2000). Recently, ten Napel et al. (2009) showed that there is indeed evidence in the distribution of SCC values that some SCC are an indication of an infected udder or quarter and others are indicative of a response to infection or a recovery from an infection. These researchers highlighted that by describing the observed distribution by a mixture of 4 normal and 1 exponential distributions provides an opportunity to distinguish between non-infected animals and animals infected with minor or major pathogens.

Using mixture models, therefore, the selection for reduced mastitis incidence may be based on the probability of mastitis given SCS, rather than selection for lowest possible SCS. More recent research has also been done to extend the ideas of Detilleux and Leroy (2000) to develop a finite mixture model for SCS, using a Bayesian approach (Ødegård et al., 2003; Gianola et al., 2004; Boettcher et al., 2007). Boettcher et al. (2007) tested four different mixture models and all were found to be more appropriate for analysis of SCS data, than the standard linear model. Moreover, although correlations of ca. 0.90 were recorded between breeding values from the mixture and linear models, changes in ranking of the higher ranked sires were reported, showing that practical benefits would be realized with the adoption of a mixture model for genetic evaluation. However, it has to be highlighted that although mixture models are potentially useful and a good alternative for analysis of SCS data, they require good data recording. Moreover, these models may be difficult to implement in practical breeding values estimations, because of computational limitations.

7. Actual situation and prospects for improvement

An accurate selection criterion must be a relevant biological trait genetically well correlated to mastitis resistance, exhibit sufficient genetic variability and have operational properties, such as easy and cheap measuring procedure on a large scale. Based on these considerations, SCC is the most widely used criterion to achieve better udder health. Repeated SCC data are indeed routinely recorded for individuals as part of milk recording schemes. Nevertheless, it is important to keep in mind that the genetic response will always be limited – as breeding objectives still favour milk quantity and content from an economic point of view.

In setting up a breeding programme, however, there are other issues that are important to take into account. Technical and infrastructural related issues, for example, are the greatest bottlenecks in genetic improvement programmes for most of the sheep farming systems. Small flock sizes, poor pedigree and performance recording, lack of clear breeding goals, lack of or poor infrastructures. These are all factors that contribute to the low participation of farmers in breeding schemes, which in turn makes achieving within-breed genetic improvement highly challenging.

Whereas artificial insemination (AI) is a common reproductive technique in dairy cattle, in dairy sheep its application is limited to experimental farms. Due to the low use of AI, the diffusion rate of a ram is from 100 to 1000 times lower than that of a bull (Carta et al., 2009). The limited use of AI, therefore, reduces the progeny group size of rams and is in general associated with poor pedigree recording, which negatively affects the accuracy of breeding value estimates (Van Vleck, 1970; Lee and Pollak, 1997). Many flocks rely on a few males, and it is not possible to know with certainty which ram is the sire of an animal. In dairy cattle, it has been reported that paternity errors can reach up to 20% of registered animals (Ron et al., 1996) and this percentage is probably even higher in sheep, drastically reducing

426 the genetic gain and the success of breeding programmes. To overcome this problem, it is
427 possible for farmers to manage natural mating by grouping ewes with a single ram (i.e.,
428 mating group) during the mating period. This management strategy would make it easier to
429 determine the correct sire of a lamb, based on the lambing date. However, the poor
430 infrastructures on the farms in general do not allow for the implementation of these strategies.
431 As an alternative, it may be possible to use DNA testing for pedigree verification or pedigree
432 assignment in cases of unrecorded mating or the use of multiple sires. Procedures have been
433 already developed for both goats and sheep (Glowatzki-Mullis et al., 2007; Rosa et al., 2013),
434 as well as dogs (DeNise et al., 2004), horses (Tozaki et al., 2001; Seyedabadi et al., 2006),
435 and cattle (Van Eenennaam et al., 2007).

436 Another problem encountered in genetic evaluation of sheep flocks is the poor genetic
437 connections between flocks, which result from the limited exchange of rams between farms.
438 This could be overcome by AI, but as discussed earlier the uptake of AI is low. This implies
439 that improvements in genetic connections need to come from exchanging rams between
440 farms. However, farmers do not see it as favourable to exchange rams between flocks, as they
441 usually think they have the best individuals. An alternative would be to implement a selection
442 scheme based on the pyramid management of the population, which is nowadays considered
443 the most efficient selection scheme for local dairy sheep (Barillet, 1997). In this scheme, the
444 nucleus flocks are at the top of the breeding pyramid. In these flocks, pedigree and milk
445 recording are implemented, and breeding value estimations are carried out to generate genetic
446 progress in these flocks. The genetic progress would be then disseminated to commercial
447 flocks through AI or natural-mating rams originated from nucleus flocks. A potential problem
448 in the implementation of this scheme is that farmers would need to be convinced regarding
449 the superior quality of the rams from the nucleus flock. However, it is likely that farmers will

450 be willing to cooperate in such a scheme once they experience the quality of the breeding
451 products. It would even be easier to realize such a scheme if it were technically or financially
452 supported by the Government, Breeder Associations or the University. The support by such
453 an Institution would reassure farmers, who sometimes just need to feel that their interests are
454 taken into account.

455 When implementing a nucleus breeding scheme, an important aspect is the genotype by
456 environment (GxE) interaction. GxE interactions could reduce the benefits for commercial
457 farmers of genetic progress generated in the nucleus flock. One of the methods used to
458 quantify GxE, is the estimation of genetic correlations (r_g) between traits measured in
459 different environments. When r_g between the phenotypic values of the same trait expressed in
460 different environments is high i.e. equal or close to 1 – then there is no GxE (Robertson,
461 1959). On the other hand, low r_g values indicate GxE, i.e. phenotypes expressed in different
462 environments are expressions of different traits. Mulder and Bijma (2005) estimated that a r_g
463 of 0.80 between two environments results in 20% less genetic gain for a trait in dairy cattle,
464 when breeding stock are selected in another environment. Mulder et al. (2006) demonstrated
465 that in dairy cattle, when r_g between environments are between 0.50 to 0.70, a single breeding
466 programme with progeny testing bulls in different environments would be optimal to breed
467 for general adaptability. However, when r_g between environments is lower than 0.50,
468 environment-specific breeding programmes are necessary to breed for specific adaptability.
469 Therefore, to realize a pyramid selection scheme for any breed, it would be important to
470 make sure that the environment of the nucleus flocks is comparable to that at the commercial
471 farms.

472 Concerning diseases and disease resistance, quantifying and accounting for the impact of
473 environmental factors is an important part of identifying and measuring true host genetic

474 variation in resistance to the disease under study. There is a risk of bias in genetic parameter
475 estimates and lost opportunities in identifying individuals with extreme genetic risk, when
476 these environmental factors are not correctly taken into account (Bishop and Woolliams,
477 2010). It is therefore necessary to determine the "optimal exposure level" in order to select
478 for mastitis resistance. Of course it would not be good to have all animals being infected;
479 however, on the other hand, if no animals are affected then there is no information upon
480 which to base the selection. It is important to realize that a lack of exposure simply means
481 that individuals do not have the opportunity to express their genetic merit for resistance, with
482 potentially highly susceptible individuals being (wrongly) classified as resistant, simply
483 because they are healthy (Bishop and Woolliams, 2010). These researchers have also
484 demonstrated that whilst true presence/absence of a disease, given exposure to infection, is
485 largely a function of the immune response, the actual prevalence of the disease and the
486 estimable genetic variation between animals will be influenced by variable exposure and the
487 sensitivity of diagnosis.

488 In implementing a breeding scheme for mastitis resistance, it has to be taken into account that
489 measurements of phenotypic indicators for mastitis resistance are time and labour intensive.
490 Therefore, the use of genetic markers to indicate resistance or susceptibility to mastitis or to
491 better exploit the phenotypic information through genomic selection (GS) is an attractive
492 proposition (Goddard and Hayes, 2007). At present, however, the available literature on GS
493 and molecular markers for mastitis resistance mainly refer to dairy cattle (Klungland et al.,
494 2001; Boichard et al., 2003; Schulman et al., 2004). In sheep, quantitative trait loci (QTL)
495 influencing SCS have recently been detected (Rupp et al., 2003b; Gutierrez-Gil et al., 2007;
496 Raadsma et al., 2009).

497 There is currently widespread excitement regarding the potential for GS to provide new
498 approaches for the improvement of sustainability traits in Holstein dairy cows. Many
499 breeding programmes worldwide have already implemented GS. However, it is important to
500 recognize that it is not obvious how GS can be implemented in small ruminant species. An
501 important limitation of applying GS to sheep, is that a reference population of considerable
502 size would be required. In dairy cattle, for example, reference populations of over 4000
503 progeny tested young bulls are available, and this scale would be difficult to achieve in sheep.
504 However, nowadays, thanks to the development of high-density SNP arrays with tens of
505 thousands of genetic markers spread across the genome, research is moving to the direction
506 of GS in sheep as well, as such arrays have also proven to be very powerful, with even a
507 small number of animals. In a GS study conducted on the Lacaune breed on three traits (milk
508 yield, fat content, SCS), Duchemin et al. (2012) have demonstrated that molecular markers
509 can be effectively used to improve current selection methods. Using a reference population of
510 about 2500 proven rams and about 44000 SNP, it was reported that accuracies of GEBV for
511 males at birth can be improved from +18 to +25%, according to the traits.
512
513 **8. Conclusions**
514 Although results reported in the literature for sheep are less frequent than for cattle, it seems
515 to be accepted that selection for reduced SCS would lead to a reduced mastitis incidence.
516 This review, however, highlights a number of elements that need to be considered when
517 setting up a breeding programme for mastitis resistance, using SCS as an indicator. Besides
518 the importance of knowledge of both genetic and environmental aspects of the traits
519 considered, the need has been stressed for having a strong and well-structured organization to
520 implement and support the programme. The heritabilities of the traits of interest, either SCS
521 or infection status, are indeed low. Therefore, it is unlikely that selection for mastitis

522 resistance by the farmers on their own will be successful. However there is a good prospect
523 for genetic improvement at farm level, when reliable pedigree and performance recording is
524 implemented across flocks and combined with breeding value estimation. This system
525 requires cooperation between the farmers and technical support from an independent
526 organisation.

527

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