Non-nutritional factors affecting lactation persistency in dairy ewes: a review

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ABSTRACT

Milk production is largely related to the shape of the lactation curve. Key elements of the lactation pattern are peak yield, which is the maximum daily yield reached during lactation, and lactation persistency, which is the medium rate of milk yield decrease after the lactation peak. The ideal lactation curve should have a reasonably high peak and a flat trend afterwards. A more persistent lactation is desirable because it is related to better animal health and reduction of feeding costs. Effective strategies to improve lactation persistency require a deep understanding of the main factors that affect this trait, including genetics, hormonal status and administration, udder morphology, seasonal changes, management, animal health (e.g. mastitis), stress and nutrition. This review covers the effects of non-nutritional factors on lactation persistency in dairy sheep.

Key Words: Dairy sheep, Lactation persistency, Genetic improvement, Milk.

RIASSUNTO

INFLUENZA DI FATTORI NON-NUTRIZIONALI SULLA PERSISTENZA DELLA LATTAZIONE NELLA PECORA DA LATTE: UNA REVIEW

La produzione di latte dipende largamente dalla forma della curva di lattazione i cui elementi chiave sono la produzione al picco, che rappresenta la produzione massima nel corso della lattazione, e la persistenza di lattazione, che è il tasso medio di riduzione di produzione di latte dopo il picco. Gli animali “pERSISTENTI” sono quelli che presentano una minore pendenza della curva di lattazione nella sua fase discendente, per cui la curva di lattazione ideale è quella con un elevato picco e un trend piatto dopo il picco. Curve di lattazione a maggiore persistenza sono preferibili per la migliore salute degli animali e per la riduzione dei costi di alimentazione. La predisposizione di interventi razionali per il miglioramento della persistenza implica la conoscenza di tutti i fattori in grado di condizionarla. Fra questi i più rilevanti sono i fattori genetici, ormonali, morfologici della mammella, stagionali, gestionali, sanitari (in particolare della mammella), di stress e alimentari. Questa rassegna prende in esame l’influenza di fattori non-nutrizionali sulla persistenza di lattazione delle pecore da latte.

Parole chiave: Pecore da latte, Persistenza di lattazione, Miglioramento genetico, Latte.
Introduction

Milk production is largely related to the shape of the lactation curve. Key elements of the lactation pattern are peak yield, which is the maximum daily yield reached during lactation, and lactation persistency, which is the rate of milk yield decrease after the lactation peak. “Persistent” animals are those with flatter lactation curves.

Domesticated animals have lactation curves with higher peaks and persistency, and thus higher milk yield, than their wild ancestors. Dairy breeds have a greater persistency of lactation than meat and wool breeds (Figure 1).

In practice, the ideal lactation curve has a high peak and a moderately flat trend afterwards. More persistent lactation is desirable because it is related to better animal health and reduction of feeding costs (Sölkner and Fuchs, 1987; Pryce et al., 1997; Dekkers et al., 1998; Grossman et al., 1999). Animals with very high peak yields are not able to consume adequate amounts of nutrients in the first part of lactation. This causes negative energy balances, reduces reproductive efficiency and increases susceptibility to diseases (Swalve, 2000; Jakobsen et al., 2002). By contrast, animals with lower peak yields but flat curves are less subject to metabolic stresses in early lactation and have a more constant pattern of energy requirements throughout lactation. This reduces feeding costs (Sölkner and Fuchs, 1987; Dekkers et al., 1998). In any case, in animals with similar peak yields, the greater the lactation persistency, the greater the total milk yield during lactation.

A proper definition of strategies to improve lactation persistency requires knowledge of the several factors that affect this trait, including genetics, hormonal status and administration, udder morphology, seasonal effects, management techniques, animal health (e.g. mastitis), stress and nutrition.

Non-nutritional factors affecting lactation persistency of dairy ewes are reviewed in this paper.
**Physiological factors affecting lactation persistency**

The pattern of the lactation curve is influenced by the number of secretory cells in the mammary gland and by the synthetic activity of each secretory cell. Growth and differentiation of the glandular epithelium during puberty and pregnancy are important determinants of the total volume of secretory epithelium and consequently of milk yield. After parturition, the maintenance of the secretory epithelium is the key factor in determining lactation persistency and total milk yield. Maintenance of milk synthesis and secretion is controlled by a combination of both systemic and local regulatory factors, as recently reviewed by Akers (2006).

**Systemic factors**

Hormones such as growth hormone (GH) and prolactin (PRL) are systemic factors involved in maintaining lactation in lactating sheep (Hooley et al., 1978). The oxytocin (OT) may also be involved in mammary cell maintenance and metabolism, as well as in myoepithelial cell contraction and milk letdown (Zamiri et al., 2001).

After the lactation peak, GH (Akers, 2002) and PRL (McMurtry et al., 1975) levels decrease. This reduces milk synthesis. “Traditional binding assays showed very little evidence for the presence of receptors of these hormones in mammary microsomes, but studies using sensitive PCR techniques or immunocytochemistry indicate that GH receptors are present” (Akers, 2006). However, the positive effect of GH on milk yield is mostly indirect by stimulating the synthesis and secretion of insulin-like growth factor-I (IGF-I). IGF-I is mainly synthesized in the liver, but it is produced and acts in other tissues also, such as the mammary parenchyma. IGF-I receptors have been identified in the mammary glands of sheep (Akers, 2002). GH administration increases IGF-I in blood, which means that GH may help the mammary epithelial cells to survive. Secretion of IGF-I is also regulated by the nutritional status of animals. For example, plasma IGF-I concentration increased when high-energy and high-protein diets were used (McGuire et al., 1992). Increasing the frequency of concentrate feeding from one to three times a day, or improving the quality of forage, increased IGF-I plasma concentrations in ewes in late pregnancy (Chestnutt and Wylie, 1995). GH treatments are an effective way of increasing milk yield, as discussed later.

The role of PRL in milk synthesis is probably related to the fact that it inhibits mammary apoptosis by suppressing the actions of IGF binding protein (IGFBP-5), which antagonizes the effects of IGF-I on the survival of mammary epithelial cells (Tonner et al., 2000). In rats, a reduction in blood PRL concentration reduced milk yield and caused a 20-25% loss in the number of secretory cells within 48 h (Flint and Knight, 1997). In sheep, administration of bromocriptine, an alkaloid that inhibits the release of PRL, 10 days after parturition reduced milk yield by 60-70% (Burvenich et al., 1991). However, administration of PRL to dairy cows has little effect on milk production or composition (Plaut et al., 1987).

**Local factors**

Local control of milk secretion is directly linked to the physical removal of the milk. The impact of these factors on the mammary function in dairy animals is evident from the known positive effects of frequency of milk removal and the negative effects of milk stasis in the mammary cistern on milk yield. The accumulation of milk in the mammary gland accelerates the involution process and reduces lacta-
tion persistency.

Local factors involved in the control of milk secretion were demonstrated in half-udder experiments carried out in cows (Stelwagen and Knight, 1997), goats (Wilde and Knight, 1990) and sheep (Nudda et al., 2002a), in which unilateral alteration of the frequency of milking affected only the treated gland. Increasing milking frequency from 1 to 2 times per day in one udder increased its milk yield without effecting the milk yield of the other udder which continued to be milked twice a day (Figure 2).

Wilde et al. (1987) identified the local factor involved in the reduction of milk secretion as a peptide, which they called feedback inhibitor of lactation (FIL). It is synthesized in the mammary epithelial cells and secreted with the milk in the alveoli. As time from milking increases, milk accumulates in the alveoli, as does this peptide. This causes a progressive reduction in milk synthesis and secretion. Thus, frequent removal of milk (and consequent of the FIL) from the mammary gland reduces local inhibitory effects on milk synthesis.

Further evidence of the existence of local factors in the mammary gland was obtained in one of our experiments where one udder half was dried, while the other continued to be milked twice a day. The milk yield of the milked udder half was 50% lower than the milk yield obtained from ewes in which both udder halves were milked twice a day (618 vs 1221 g/d) (Cannas et al., 2002).

It has been hypothesized that there is a proteolytic casein fragment in the mammary gland which inhibits milk synthesis (Silanikove et al., 2000). This peptide, which is made up of residues 1-28 of \( \beta \)-casein produced by the proteolytic activity of plasmin, reduces milk secretion in goats and cows. In goats, injection of casein hydrolyzates into the udder caused a local inflammation and a loss of the integrity of the tight junction (TJ), followed by a rapid drying off of the gland (Shamay et al., 2002). This finding was also supported by our experiment simulating once a day milking (1X) in the same half-udder (Pulina et al., 2005). In this experiment the injection of casein hydrolyzates into the mammary gland of goats caused a reduction in milk yield, and an increase in somatic cell count (SCC), plasmin and Na in milk.

Figure 2. Milk production (kg/d) of right (●) and left (○) udder halves milked once or twice a day.

The right udder halves were milked twice a day for the first period of the experiment and once a day in the second period. The left udder halves were milked once a day in the first period and twice a day in the second period (Nudda et al., 2002a).
Other factors: the role of the plasmin-plasminogen system

Plasmin is the predominant protease in milk and is mainly associated with casein micelles, which are its substrate of action. Plasmin is responsible for the hydrolysis of \( \alpha \) and \( \beta \) casein in milk. Plasmin and its precursor, plasminogen (PG), are present simultaneously in milk. The PG is converted into active plasmin by the action of the plasminogen-activator (PA), whose activity is reduced by PA inhibitors (PAI) (Politis, 1996). The plasmin-plasminogen system seems to be involved in the events that occur during the gradual involution of the mammary gland (Politis, 1996). Indeed, the activity of PG and plasmin usually increases in milk as lactation progresses even if in Comisana ewes plasmin and PG activities were higher in the high SCC milk than in the low SCC milk regardless of stage of lactation (Albenzio et al., 2004).

IGF-I, which acts as a mediator of the GH, and good nutritional status of the animals also help to decrease PA, probably through the stimulation of PAI (Padayatty et al., 1993). It is well known that administration of exogenous GH to sheep (Baldi et al., 1997; Baldi, 1999; Chiofalo et al., 1999), cows (Politis et al., 1990), and goats (Baldi et al., 2002) increases milk yield and lactation persistency and reduces plasmin activity, probably through its mediator IGF-I.

Disruption of tight junctions integrity

The involution of mammary secretory cells is triggered by the disruption of the TJ structures, which encircle the cells and fuse adjacent cell membranes, thus forming a barrier between blood and milk. The TJ are connected with the cytoskeleton, a network of micro-filaments that is probably involved in the secretion of the neo-synthesized milk components from the secretory cells into the alveolar lumen. During lactation, or in conditions in which the integrity of TJ is maintained, milk precursors reach the alveolar lumen by passing through the secretory cells (the transcellular route). During involution (but also in other conditions such as pregnancy, mastitis, and extended milking intervals) the TJ become leaky and allow the passage of some blood components between cells that then reach the alveolar lumen (the paracellular route). As a consequence, TJ leakiness affects cytoskeleton activity, reducing its dynamic properties in the transfer of neo-synthesized milk components towards the apical membrane of the mammary secretory cells (Mepham, 1987). The reduced secretion of milk components inhibits further synthesis and makes the involution of secretory cells more likely.

The impairment of TJ, which causes the activation of the paracellular pathway, allows the passage of substances between epithelial cells, causing an increase of Na in milk and the passage of lactose into the blood (Stelwagen et al., 1994). A high Na/K ratio in milk has been associated with the mechanisms that reduce milk yield in cases where the permeability of mammary TJ is increased (Allen, 1990).

The TJ can be damaged by: the increased activity of plasmin, as lactation progresses, in case of mastitis or prolonged milking intervals; the massive migration of somatic cells (leukocytes or white blood cells) from blood to mammary gland to defend its tissues from pathogens in case of inflammations (mastitis); and the stretching caused by excessive accumulation of milk (Mepham, 1987) with long milking intervals (Stelwagen et al., 1994).

Therefore, it appears that mammary involution is controlled by local and systemic factors with highly integrated mechanisms of control.
Genetics

The genetic modification of the shape of the lactation curve in an economically desirable direction is an interesting challenge for scientists and professionals of the dairy industry (Rekaya et al., 2001). In dairy cattle, several studies have investigated the relationships between fundamental traits of lactation curve shape, such as persistency and peak yield, and productive and functional traits. However, the strategies to genetically improve lactation persistency are not very clear. At present, the lack of consensus on the most suitable measure of persistency is a major constraint. The several approaches proposed in the literature (Solkner and Fuchs, 1987; Gengler, 1996; Jamrozik et al., 1998; Grossman et al., 1999; Togashi and Lin, 2003) have been based on: I) ratios between cumulated yields of different stages of lactation; II) variability of test day yields; III) parameters of mathematical models of lactation curves; and IV) days in which a constant level of production is maintained. As a result, a wide range of estimated values for genetic parameters of lactation persistency is found in the literature, depending on the measure used to define this trait. For example, heritability goes from a value of around zero to values higher than 0.30. Some measures of persistency are highly correlated with total lactation yield, even though some authors state that a robust measure of persistency should be independent from total yield (real persistency) (Gengler, 1996) or that the total lactation yield should be included as a (co)variate in the genetic model used to estimate genetic parameters and breeding values for lactation persistency (Swalve, 1995). In any case, most scientists agree that persistency possesses a certain degree of genetic variation, with moderate heritability (0.15-0.20) (Macciotta et al., 2006), and that selection for this trait is feasible.

Genetic aspects of the shape of the lactation curve have been little investigated in dairy sheep. At present, in this species the main breeding goal is total lactation yield, while in only a few breeds are milk composition traits considered (Barillet, 1997; Macciotta et al., 2004). Selection based on lactation curve traits is also limited by the reduced number of milk test day (TD) records available. However, since Mediterranean dairy sheep farming system is often characterized by a low level of inputs (feed, technology, equipments), genetic improvement of traits that affect the economic efficiency of the animal by reducing costs rather than increasing production (Groen et al., 1997), such as lactation curve shape traits, could be considered as an interesting alternative.

Chang et al. (2001, 2002) have investigated the genetic variation of lactation curve shape in sheep by using a quadratic function and the model of Wood (1967). Heritability ranges were 0.23-0.35, 0.15-0.35 and 0.17-0.27, respectively, for parameters \(a\), \(b\) and \(c\) of the Wood’s model (the third parameter controls the descending rate of the curve after the lactation peak, i.e. lactation persistency). This indicates that the lactation curve shape in sheep can be altered by selection on the basis of parameters of lactation curve functions.

A multivariate measure of lactation persistency has been proposed for dairy sheep (Macciotta et al., 2003). In this approach, TD milk yields recorded at different times from parturition are considered different traits and are analysed with the multivariate factor analysis technique.

In the factor analysis approach, the correlation matrix of original variables \(S\) is decomposed as

\[ S = BB' + \Psi \]

where \(B\) is the matrix of the factor coefficients, i.e. of the correlations between the
new latent variables and the original variables, and $\Psi$ is a residual correlation matrix. Factor analysis is able to extract from original data new latent variables (factors) that are able to reconstruct a relevant quota of the variability of original variables. In contrast with all the previously reported measurements, this multivariate approach does not require an *a priori* definition of what persistency is, because the new factors are objectively derived from the correlation matrix among the original variables.

The B matrix obtained by applying factor analysis on milk TD records of 380 Sarda breed dairy ewes is shown in Table 1. Each ewe had 5 TD records, which were considered as different traits. Two common factors explained about 73% of the original variance. Factor 1 is associated with the TD of the last part of lactation and can be considered an indicator of lactation persistency, whereas Factor 2 is correlated with the TD of the first part of lactation, and can be considered an indicator of production levels in early lactation. The relationships between Factor 1 scores and lactation curve shape can be inferred from Figure 3 where the average lactation patterns of five different classes of animals, grouped according to Factor 1 scores, are shown. As the values of Factor 1 increase, the persistency of lactation tends to increase. A mixed model analysis of Factor 1 scores gave a repeatability value of 0.32, which agrees with previous results reported for dairy cattle (Gengler, 1996). Factor 1 scores were affected by parity and year of lambing, i.e. sources of variability that are known to affect lactation persistency.

### Use of hormones

**Somatotropin**

It is well known that exogenous somatotropin (ST) increases milk production in cows and in other dairy ruminants (Akers, 2006). In general, ST administration to dairy ruminants increases milk production in the short term (the immediate post-injection period) and has a medium to long term positive effect on lactation persistency also (Baldi, 1999). For example, the administration of 320 mg of bovine ST to Comisana ewes increased significantly milk yield (Figure 4) (D’Urso et al., 1998). The ST administration also increased milk yield by 10-40% in cows (Flint et al., 2005) and by 14-29% in dairy goats (Baldi, 1999).

In several trials, dairy ewes treated with ST during pregnancy, early-mid lac-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD1</td>
<td>0.20</td>
<td>0.85</td>
</tr>
<tr>
<td>TD2</td>
<td>0.44</td>
<td>0.82</td>
</tr>
<tr>
<td>TD3</td>
<td>0.65</td>
<td>0.53</td>
</tr>
<tr>
<td>TD4</td>
<td>0.84</td>
<td>0.30</td>
</tr>
<tr>
<td>TD5</td>
<td>0.70</td>
<td>0.19</td>
</tr>
<tr>
<td>Variance explained</td>
<td>0.37</td>
<td>0.36</td>
</tr>
</tbody>
</table>
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Lactation curves for the milk yield of animals with different classes of factor 1 (Macciotta et al., 2003).

Figure 3.

The lactation responses of dairy ewes to exogenous ST are higher than those usually observed in dairy cows. This could be explained, at least partially, by the fact that the genetic selection for milk production has been less intense in dairy ewes than in dairy cows (Cannas, 1996).
The role of ST in sheep has also been recently investigated by producing ST transgenic sheep with doubled levels of ST in plasma. The gains in productivity were counterbalanced by a decrease in reproductive efficiency and an increase in several disease problems, which became more evident as the animals aged (Adams and Briegel, 2005).

Oxytocin
The daily injection of OT (2 IU) in Mehraban ewes starting from 15 days postpartum increased the lactation length by 30 days compared to the control group (Zamiri et al., 2001). The amount of milk recorded during the entire lactation was 55% higher for the OT treated group than for the control group. In this study the parameters of the lactation curve were not estimated. However, in a similar experiment in dairy cows (Nostrand et al., 1991), the OT group produced 849 kg more milk during the whole lactation period.

Table 2. Response of dairy ewes to exogenous somatotropin (ST) administration in pregnancy and early-mid lactation.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lactation stage</th>
<th>oST or bST dose*</th>
<th>Milk Yield increase (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assaf</td>
<td>after peak</td>
<td>0.1 mg oST/kg BW</td>
<td>+55.5</td>
<td>Leibovich et al., 2001</td>
</tr>
<tr>
<td>Manchega</td>
<td>weeks 3-8</td>
<td>80 mg bST/14 d</td>
<td>+20.2</td>
<td>Fernandez et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>160 mg bST/14 d</td>
<td>+34.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>240 mg bST/14 d</td>
<td>+30.2</td>
<td></td>
</tr>
<tr>
<td>Comisana</td>
<td>62 days</td>
<td>High starch and</td>
<td>+20.6</td>
<td>Dell’Orto et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>320 mg bST/head</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low starch and</td>
<td>+35.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>320 mg bST/head</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arcott</td>
<td>pregnancy</td>
<td>0.1 mg bST/kg BW</td>
<td>+41.9</td>
<td>Stelwagen et al., 1993</td>
</tr>
</tbody>
</table>

*oST = ovine somatotropin, bST = bovine somatotropin.
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lactation and showed a greater lactation persistency than the control group.

Luteinizing hormone

Ewes superovulated with pregnant mare serum gonadotropin had 31% better developed mammary glands at parturition and 55% higher milk production during the first 12 weeks of lactation (Frimawaty and Manalu, 1999). This is because superovulation prior to mating increases corpora lutea number and mean serum progesterone concentration during pregnancy (Manalu et al., 1998). Analysis of the mammary glands at the end of lactation showed that superovulated ewes had 79% higher total DNA and 56% higher total RNA than non-superovulated ewes (Manalu et al., 2000), indicating a higher number of secretory cells and higher synthetic activity per cell.

Lambing season, parity and type of lambing

The effects of lambing season on persistency of lactation have been mainly attributed to seasonal differences in the availability and quality of pasture (Cappio-Borlino et al., 1997b). Ewes that lambed when the maximum amount of forage was available had a higher milk yield, probably because of a positive effect on the differentiation of udder secretory cells and on the accumulation of body reserves. Portolano et al. (1996) observed that Comisana dairy ewes which lambed in autumn had greater persistency, lower peak yield and reached lactation peak later than ewes of the same parity which lambed in winter. This phenomenon may be due to the effect of different lambing seasons on grazing management conditions. In fact, ewes lambing in autumn have their peak milk yield depressed by the effects of winter and can only take advantage of more abundant and better quality pasture in the second part of lactation.

The influence of lambing season on milk yield may also be related to photoperiod. In Mediterranean areas, ewe lactation generally occurs in the period when days are lengthening. In dairy ewes, an increase in the hours of light seemed to increase milk production and feed intake (Bocquier et al., 1997). This effect was evident when the treatment lasted more than 30 days and may be explained by the fact that animals fed more when there was more light.

Table 3. Response of dairy ewes to exogenous somatotropin (ST) administration in late lactation.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lactation stage</th>
<th>bST dose</th>
<th>Milk Yield increase (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manchega</td>
<td>weeks 11-23</td>
<td>80 mg bST/14d</td>
<td>+41.3</td>
<td>Fernandez et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>160 mg bST/14d</td>
<td>+53.2</td>
<td></td>
</tr>
<tr>
<td>Comisana</td>
<td>week 14</td>
<td>LSR + 320 mg bST/head</td>
<td>+34.0</td>
<td>D’Urso et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSR + 320 mg bST/head</td>
<td>+42.4</td>
<td></td>
</tr>
<tr>
<td>Comisana</td>
<td>200 days</td>
<td>120 mg bST/21 days</td>
<td>+21.9</td>
<td>Chiofalo et al., 1999</td>
</tr>
</tbody>
</table>

*bst = bovine somatotropin.

bLSR = low stocking rate.

bHSR = high stocking rate.

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Differently, sheep submitted to a sharp increase in day length for a short period produced less milk (Pulina et al., 2002).

Analysis of the evolution of the shape of the lactation curve according to the number of lambings showed that Lacaune (Barillet, 1985), Laxta (Gabiša et al., 1993), Sarda (Carta et al., 1995) and Valle del Belice (Cappio-Borlino et al., 1997b) dairy ewes produced more milk after the third or subsequent parities (Figure 5). By contrast, the peak yield took place quite late and lactation was more persistent in primiparous ewes of almost all dairy breeds. Stanton et al. (1992) observed the same effect in dairy cows and suggested that this pattern could be due to the fact that the body and mammary gland of young animals are still developing during the first part of lactation. In sheep, this effect is evident only in the first part of lactation up to 70-120 days in milk (DIM), after which it gradually becomes less pronounced; thus, the rest of the 1st lactation curve becomes similar to that of pluriparous ewes (Cappio-Borlino et al., 1997a; Ruiz et al., 2000). No differences in lactation persistency has been observed in Comisana ewes with different parity (Sevi et al., 2000).

Several studies have reported higher milk yields for ewes with multiple births in both non-dairy and dairy breeds (Table 4 and Figure 6). This can be due to the fact that ewes rearing multiple fetuses or with a heavy single fetus have higher placental weight, serum progesterone and placental lactogen hormones during pregnancy than single birth ewes without heavy fetuses.

Table 4. Effect of prolificacy on milk yield in different sheep breeds.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Purpose</th>
<th>Milk Yield twins vs single</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delle Langhe</td>
<td>Milk</td>
<td>+10%</td>
<td>Ubertalle et al., 1990</td>
</tr>
<tr>
<td>Sarda</td>
<td>Milk</td>
<td>+11%</td>
<td>Pulina et al., 1993</td>
</tr>
<tr>
<td>Churra</td>
<td>Milk</td>
<td>+4.5%</td>
<td>Gonzalo et al., 1994</td>
</tr>
<tr>
<td>Rambouillet, Columbia, Polypay</td>
<td>Meat-wool</td>
<td>+44%</td>
<td>Snowder and Glimp, 1991</td>
</tr>
<tr>
<td>Suffolk</td>
<td>Meat</td>
<td>+63%</td>
<td>Snowder and Glimp, 1991</td>
</tr>
<tr>
<td>Merino</td>
<td>Meat-wool</td>
<td>+46-51%</td>
<td>Sokolov and Kuts, 1984</td>
</tr>
</tbody>
</table>
Higher average serum progesterone levels during pregnancy are related to well developed mammary glands at parturition, which show a high number of mammary cells and increased synthetic activity (Manalu et al., 1998, 2000). In addition, since the mammary glands are suckled more frequently by twins than by singles, the local inhibitors of milk secretion, such as the FIL, are removed. Recently, Canu et al. (2006) observed that in Sarda dairy sheep milk yield increased linearly as prolificacy increased (kg/year head\textsuperscript{1} 136 \textit{vs} 150 for prolificacy<14% and >28%, respectively), both in organic and conventional farms.

**Weaning system**

Reduction of the suckling period during lactation is a widespread practice in dairy animals. This is done to increase the length of the milking period and the amount of marketable milk. However, particular attention has to be paid to the weaning technique used, because it can affect milk yield after weaning.

In studies on different weaning systems carried out during the first 30 days of lactation, ewes were: machine milked twice daily after weaning at 24 hours post partum (D1), lamb suckled for 30 days and then machine milked twice a day after weaning (D30), or suckled for part of the day and then separated from their lambs during the night to allow machine milking once daily the following morning (MIX) (McKusick et al., 1999, 2001). Total commercial milk production of MIX ewes was only 10% lower than that of D1 ewes (236 \textit{vs} 261 kg/ewe per lactation) and 37% higher than that of D30 ewes (172 kg/ewe per lactation). Average lactation length (suckling + milking period) of the various weaning systems was similar (about 183 ± 5 d). McKusick et al. (2002), comparing the
MIX and D1 weaning systems in East Friesian crossbreed ewes, found a higher milk yield in MIX ewes 2 and 4 weeks post-partum. This was probably due to a more frequent and complete udder evacuation by the suckling lambs than by machine milking, as the latter reduces local concentrations of the FIL. In that study, a clear effect of weaning system on persistency was not observed.

In another study on East Friesian ewes, Thomas et al. (2001) observed that raising lambs on milk replacer and starting milking the ewes 24-36 hours after parturition increased milk production by 61% compared to starting machine milking after the lambs were weaned at 30 days of age.

**Milking frequency**

The reduction of milking frequency or the extension of milking intervals can accelerate the involution process of the mammary gland and reduce lactation persistency through a mechanism that involves systemic and local factors, as described previously in this paper.

The effect of the reduction of daily milking frequency from two (2X) to one (1X) is similar in dairy and non-dairy breeds (Table 5) (Pulina and Nudda, 1996). For higher frequencies, however, the effect is greater on non-dairy ewes than in dairy ewes (Bencini, 1993): the increase of milking frequency from 2X to three times per day (3X) resulted in a increase of milk yield of 3% and 21% in Sarda (Cannas et al., 1991) and Merino ewes (Bencini, 1993), respectively. This difference is probably due to the different udder storage capacity of the two breeds. The response to an increase in milking frequency is not constant across breeds: in a study carried out on East Friesian crossbreed ewes 25% of the ewes subjected to a 3X did not show any response, 50% of them produced 13% more milk, and 37.5% of the ewes produced 36% more milk during the first 30 days of lactation (De Bie et al., 2000). It is worth pointing out that when the third milking was removed, the milk yield dropped immediately to the level of 2X (De Bie et al., 2000). Thus, the third milking at the beginning of lactation created a higher lactation peak, but the positive effect was not maintained during the rest of lactation, probably because of a limited cistern size.

When one evening milking per week was removed, milk yield was reduced by 7.0% in Poll Dorset (Knight and Gosling, 1995), 8.5% in Manchega (Huidobro, 1989), 14% in Sarda (Casu and Boyazoglu, 1974) and 25.6% in Prealpes du Sud (Labussière et al., 1974) ewes. The magnitude of the effect of omitting an evening milking may also be related to the production level and the cistern size of the animals. Castillo et al. (2005) evaluated the effects of 1X versus 2X on milk yield in Manchega (medium yielding) and Lacaune (high yielding) dairy ewes in two different stages of lactation: early-mid and mid-late lactation. The reduction in milk yield when one milking per day was omitted in early-mid lactation was higher in the Manchega (-33%) than in the Lacaune (-10%) breed. The authors attributed the result to the lower cistern storage capacity of Manchega ewes (63%) compared to Lacaune ewes (77%).

**Udder morphology and cistern dimension**

Since the alveoli are the site of action of the inhibitor peptides (Henderson and Peaker, 1984), the local inhibitory factors (i.e. the FIL) affect the rate of secretion when the milk is stored in the secretory tissue, whereas they are inactive in the milk stored in the cistern. As a consequence, the action of the FIL should be less strong in animals with
larger cisterns, because a large proportion of the milk is stored in the mammary cistern and so the time during which the milk is in contact with the alveoli is reduced. Some studies have shown that milk production is positively influenced by mammary gland size (Labussière et al., 1981; Bencini, 1993) and cistern dimension (Nudda et al., 2000; Rovai et al., 2002). Studies using ultrasound techniques to measure cistern size showed a strong positive relationship between cistern dimension and milk yield in Sarda ($r = 0.74; P<0.001$; Nudda et al., 2002b) and Manchega ewes ($r = 0.76; P<0.01$; Rovai et al., 2002). The hypothesis of a weaker action of the FIL in animals with larger cisterns was tested in an experiment comparing dairy and non-dairy breeds (Nudda et al., 2002a). Two breeds highly selected for milk production (Sarda and Awassi) produced 18% to 24% less milk when milking frequency was reduced from twice to once a day. Similar results were observed in Merino ewes, a wool breed not selected for milk production (Nudda et al., 2002a). This result is probably due to the fact that both cistern size and average yield of Merino ewes were smaller, and thus the ratios between milk volume and milk cistern storage capacity were similar in dairy and non-dairy breeds. In the same trial, the reduction of milk yield observed in once per day milking increased in proportion with the production level of Sarda ewes, while this reduction was independent of the production level in Merino ewes. This was probably because the latter produced very little milk.

**Stress**

The behaviour of animals in the milking parlour is probably influenced by both genetic factors and the previous handling experience of the animals. The stress

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**Table 5. Influence of milking frequency on milk yield (MY) in dairy and non-dairy ewes (Pulina and Nudda, 1996).**

<table>
<thead>
<tr>
<th>Breed</th>
<th>Milk yield kg/d</th>
<th>MY variation in % compared to 2X&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chios</td>
<td>0.891</td>
<td>-21.6</td>
<td>Papachristoforou et al., 1982</td>
</tr>
<tr>
<td>Churra</td>
<td>0.803</td>
<td>-47.0</td>
<td>Purroy Unanua and Díaz, 1983</td>
</tr>
<tr>
<td>Comisana</td>
<td>0.387</td>
<td>-26.4</td>
<td>Battaglini et al., 1979</td>
</tr>
<tr>
<td>Comisana</td>
<td>0.440</td>
<td>-21.3</td>
<td>De Maria Ghionet et al., 1982</td>
</tr>
<tr>
<td>Lacaune</td>
<td>0.933</td>
<td>-41.0</td>
<td>Labussière et al., 1983</td>
</tr>
<tr>
<td>Meat sheep breed</td>
<td>1.430</td>
<td>-20.0</td>
<td>Morag, 1968</td>
</tr>
<tr>
<td>Pol Dorset</td>
<td>0.494</td>
<td>-7.3</td>
<td>Knight and Gosling, 1995</td>
</tr>
<tr>
<td>Prealpes du Sud</td>
<td>1.008</td>
<td>-51.3</td>
<td>Labussière et al., 1974</td>
</tr>
<tr>
<td>Sarda</td>
<td>1.177</td>
<td>-8.8</td>
<td>Enne et al., 1972</td>
</tr>
<tr>
<td>Sarda</td>
<td>1.568</td>
<td>-37.0</td>
<td>Cannas et al., 1991</td>
</tr>
<tr>
<td>Tsigai</td>
<td>0.562</td>
<td>-65.0</td>
<td>Mykus and Masar, 1978</td>
</tr>
</tbody>
</table>

<sup>a</sup>1X, 2X and 3X correspond to once-, twice- and three times daily milking, respectively.
caused by fear of humans has practical implications on the dairy animals productivity performances. Therefore, reducing the emotional or physical stress of dairy animals helps to increase their productivity and maintain their health status.

Dimitrov-Ivanov and Djorbineva (2002) found that machine-milked calm ewes produced more milk than nervous ones (Table 6). In cattle, animals with previous experience of quiet handling became calmer and easier to handle in the future. The presence of a rough handler did not modify the total milk yield per milking of dairy cows but increased their residual milk by 70% (Rushen et al., 1999), thus increasing milking duration. This can be explained by inhibition of oxytocin release by catecholamines, which are released from the adrenal gland in response to many types of stress, including fright (Bruckmaier and Blum, 1998).

In primiparous ewes, machine milked 8h after lambing, baseline levels of adrenaline and noradrenaline were slightly higher on day 1 than on day 15 of milking; and baseline levels of cortisol were significantly influenced by day of lactation (Negrão and Marnet, 2003). Higher levels of these hormones on day 1 were probably influenced by parturition. After the initial stress, OT and milk ejection increased gradually, suggesting that most ewes had adapted to machine milking by day 15.

In a study of Rassu et al. (2006), primiparous dairy ewes that started to enter the milking parlour 1 week before lamb weaning showed a significantly lower milk SCC on the first 3 days of machine milking in comparison with untrained ewes. The lowest content of milk fat was found on the first day of milking for both groups. Blood cortisol levels were not affected by the treatments during the study period (i.e. until 10 DIM). The authors hypothesized that a week of training in the machine parlour was not long enough to allow a reduction of the stress caused by machine milking and weaning in primiparous ewes.

Two main mechanisms may be involved in the response of animal productivity to stress: a local mechanism, proposed by Silanikove et al. (2000), which connects the plasmin-plasminogen system to the autocrine inhibition of lactation; and a systemic mechanism which takes

<table>
<thead>
<tr>
<th>Functional parameters</th>
<th>Calm</th>
<th>Nervous</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total morning milk</td>
<td>592</td>
<td>477</td>
<td>**</td>
</tr>
<tr>
<td>Machine milk</td>
<td>421</td>
<td>336</td>
<td>*</td>
</tr>
<tr>
<td>Machine steering milk</td>
<td>38.3</td>
<td>34.8</td>
<td>ns</td>
</tr>
<tr>
<td>Hand steering milk</td>
<td>137.1</td>
<td>107.4</td>
<td>*</td>
</tr>
<tr>
<td>Machine Milking Time</td>
<td>31.4</td>
<td>27.4</td>
<td>*</td>
</tr>
<tr>
<td>Milk flow rate</td>
<td>15.6</td>
<td>13.6</td>
<td>*</td>
</tr>
<tr>
<td>Milk ejection latency</td>
<td>1.9</td>
<td>5.3</td>
<td>***</td>
</tr>
</tbody>
</table>

*: P<0.05; **: P<0.01; ***: P<0.001
into account the role of the hypothalamic-pituitary-adrenal (HPA) axis in determining the rate of milk secretion (Matteri et al., 2000).

Silanikove et al. (2000) showed that stress activates the HPA axis that liberates cortisol into blood plasma. This in turn induces the liberation of the PA from the mammary epithelial cells into the mammary cistern where it activates the plasmin system that degrades β-casein and produces the residue 1-28 β-casein. This is also called proteoso-peptone channel blocking (PPCB). PPCB inhibits the ion channels in mammary epithelia apical membranes and thus also inhibits lactose and monovalent ion secretion. This results in a decrease in milk volume (Figure 7). When injecting the 1-28 β-casein fraction into the udder lumen of goats, the authors observed a transient reduction in milk production, which was not associated with the disruption of the integrity of the mammary cell junctions.

In the systemic mechanism, stress activates the HPA axis: the response to different stress factors causes initially a release of the hypothalamic factors vasopressin and corticotropin releasing hormones, which stimulate the secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland. The ACTH stimulates the synthesis and release of glucocorticoids (cortisol and corticosterone) from the adrenal cortex. The main function of cortisol, which is secreted within a few minutes after exposure to stress, is to mobilize energy reserves to promote hyperglycemia and reduce cellular glucose uptake (Borski, 2000). In dairy animals, cortisol causes a decrease of milk synthesis, by blocking the uptake of glucose by the mammary gland (Davis and Collier, 1985). Simulation of stress using ACTH treatment in dairy cows led to a substantial increase in cortisol concentrations and a reduction of mammary TJ leakiness (Stelwagen et al., 1998), with an involution of the mammary gland (see below). A secondary effect of stress is the inhibition of PRL synthesis by the pituitary gland, due to the hypothalamic release of dopamine. Both situations cause a transient metabolic energy surplus, due to a reduction in the energy output by the milk and an increase in mobilization of stored energy. This is caused by a sharp increase in cortisol concentrations.
LACTATION PERSISTENCY IN DAIRY EwES

Increased levels of glucocorticoids, followed by an increase in insulin and adipose tissue uptake capacity. If the stress level remains, it may have negative effects on lactation persistency, especially in the second half of lactation, due to increased secretion of the leptin hormone by adipose tissue; this hormone inhibits the positive IGF-I action on mammary parenchyma (Silva et al., 2002). In fact, Cannas et al. (unpublished data) found a negative relationship between leptin concentration in the blood and milk yield in ewes with different DM intake levels (Table 7). However, recently Thorn et al. (2006) found that leptin does not act directly on bovine mammary epithelial cells, because such cells showed a negligible leptin receptor expression in vitro.

Table 7. Milk yield, milk fat content and DM intake in Sardinian breed ewes classified according to the blood leptin content (experimental data obtained by Cannas et al.).

<table>
<thead>
<tr>
<th>Leptin class ng/ml</th>
<th>Leptin ng/ml</th>
<th>Milk yield kg</th>
<th>Fat %</th>
<th>FCM* kg</th>
<th>DM Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.30</td>
<td>1.95</td>
<td>1.983</td>
<td>6.90</td>
<td>2.044</td>
<td>2.79</td>
</tr>
<tr>
<td>&gt;2.30</td>
<td>2.70</td>
<td>1.434</td>
<td>7.28</td>
<td>1.531</td>
<td>2.22</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.039</td>
<td>0.490</td>
<td>0.034</td>
<td>0.088</td>
</tr>
</tbody>
</table>

*FCM = Fat corrected milk

Mastitis

Although clinical mastitis decreases milk yield, subclinical mastitis in sheep is economically more important, because it is more frequent (Ruiu and Pulina, 1992) and it is associated with a decrease in milk production, quality and coagulation properties (Nudda et al., 2001; Albenzio et al., 2002). The coagulase negative staphylococci are the most prevalent pathogens in the mammary gland of sheep (Gonzalo et al., 2002; McDougall et al., 2002). Bacterial infection of the mammary gland is associated with higher SCC in milk (Figure 8) (Pulina et al., unpublished data). On the other hand, the occurrence of subclinical mastitis may not be accompanied by the isolation of the

Table 8. Least square means of somatic cell count (SCC) and milk yield losses (1322 Churra ewes; 9592 milk samples) (Gonzalo et al., 2002).

<table>
<thead>
<tr>
<th>Infection status</th>
<th>SCC (x10^3/ml)</th>
<th>Milk losses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>82</td>
<td>-</td>
</tr>
<tr>
<td>Infection by minor pathogens</td>
<td>120</td>
<td>2.6</td>
</tr>
<tr>
<td>Unilateral inf. by NSCNSa</td>
<td>597</td>
<td>5.1</td>
</tr>
<tr>
<td>Unilateral infection by major pathogens</td>
<td>1317</td>
<td>8.8</td>
</tr>
<tr>
<td>Bilateral infection by NSCNS</td>
<td>1547</td>
<td>3.6</td>
</tr>
<tr>
<td>Bilateral infection by major pathogens</td>
<td>2351</td>
<td>10.1</td>
</tr>
</tbody>
</table>

aNSCNS = Novobiocin sensitive coagulate-negative staphylococci
Figure 8. Relationship between the probability of isolating microorganisms and somatic cell count (SCC) in half udders of Sarda dairy ewes (experimental data obtained by Pulina et al.)

\[ y = 16.11 \ln(x) - 77.22 \]
\[ R^2 = 0.94 \]

Figure 9. Lactation curves of Sarda dairy ewes positive and negative to the mastitis test (Pulina et al., 1993)
LACTATION PERSISTENCY IN DAIRY EWES

etiological agent probably because some enzymes may thwart pathogen detection (Albenzio et al., 2002).

The losses of milk yield through intramammary infection in sheep vary with the type of pathogen. High SCC, corresponding to major pathogens, causes larger milk yield losses than low SCC (Table 8) (Gonzalo et al., 2002).

Sarda breed ewes with mammary glands positive on bacteriological analysis suffered a reduction in total milk yield of about 24% during lactation when compared to negative animals (Figure 9). The occurrence of intramammary infection before the peak caused a reduction in peak yield and, since milk yield loss was maintained during lactation, a consequent lower persistency was also observed (Pulina et al., 1993).

Direct selection for mastitis resistance has been considered inefficient because heritability of SCC, as an indirect measurement of udder health, is low in dairy sheep (Table 9), similarly to dairy cows (Lund et al., 1994).

Cows with very low SCC levels may be more susceptible to mastitis than those with higher SCC (Kehrli and Shuster, 1994). Studies based on experimental infection of cows reported that animals resisting udder infection had higher SCC before the pathogenic infection than animals that became infected (Schukken et al., 1998). This may also be true in dairy sheep. In our observations of an experimental flock where Staphylococcus aureus were found, all milk samples from animals with clinical signs of mastitis and dry-off of the gland had low SCC (<300,000/ml). This observation, however, needs to be confirmed by analyzing a greater number of samples.

Bergonier and Berthelot (2003) proposed a method for estimating the presence of sub-clinical mastitis in ewes. In a series of checks of the same animal during lactation, an udder is considered “healthy” if every SCC (except possibly 2) is below 500,000 cells per ml, “infected” when at least two SCC are over one million cells per ml and “doubtful” between these two figures. Using this classification on 90 Sarda ewes with 6 samplings of each ewe, we classified three estimated udder health status (EUS) groups. The EUS classes significantly influenced both milk yield and persistency (Figure 10).

We modified the method of Bergonier and Berthelot (2003) by classifying the ewes in two EUS groups as follows: animals with a SCC under 7.5x10^5/ml throughout the lactation period were considered healthy (H-ewes), while those with a SCC value above 1x10^6/ml starting from the second sampling date were considered non-healthy (NH-ewes). In reality, the lower milk yield of the NH-ewes was not related to persistency, but was mainly due to a rapid loss of efficiency of

Table 9. Hereditability of somatic cell count (SCC) in milk of dairy sheep.

<table>
<thead>
<tr>
<th>Character</th>
<th>$h^2$</th>
<th>Breed</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSCSA</td>
<td>0.13</td>
<td>Lacaune</td>
<td>Rupp et al., 2002</td>
</tr>
<tr>
<td>LSCS</td>
<td>0.15</td>
<td>Lacaune</td>
<td>Barillet et al., 2001</td>
</tr>
<tr>
<td>Log SCC</td>
<td>0.09</td>
<td>Churra</td>
<td>El-Saied et al., 1998</td>
</tr>
<tr>
<td>Log SCC</td>
<td>0.14</td>
<td>Chios</td>
<td>Ligda et al., 2002</td>
</tr>
<tr>
<td>LSCS 0.12-0.16</td>
<td>0.12-0.16</td>
<td>Manchega</td>
<td>Serrano et al., 2003</td>
</tr>
</tbody>
</table>

$LSCS = Lactation somatic cell score$
Figure 10. Milk lactation curve in Sarda dairy ewes with udder classified healthy, doubtful and infected using the method of Bergonier and Berthelot (2003).

**Samplings were performed every two weeks**

Figure 11. Milk lactation curve in Sarda dairy ewes with udder classified as healthy and infected using our modification of the method of Bergonier and Berthelot (2003).

**Samplings were performed every two weeks**
synthesis of their secretory cells after the lactation peak.

**Conclusions**

Because of the economic importance of lactation persistency, it is desirable to have a flock with many ewes showing lactation curves as flat as possible.

Even though exogenous administration of hormones (i.e. ST and OT) effectively increases milk yield, we believe that more permanent and profitable results can be achieved by: a) enhancing technical practices, b) focusing on better genetic goals for dairy sheep, and c) taking care of udder health.

Genetic improvement for persistency leads to animals with high mammary storage capacity, longer lifetime of secretory cells and high levels of lactogen hormones.

Improving prolificacy increases persistency directly by increasing the population of secretory cells at the beginning of lactation and indirectly by the more complete and frequent evacuation of udder by the suckling twins.

A larger udder storage cistern makes milk loss due to less frequent milking negligible. In general, a 3X, 2X and 1X daily milking protocol can be conveniently adopted for 1-80 DIM, 80-160 DIM and >160 DIM sheep, respectively. However, milk yield gains/losses over the continuous 2X routine must be carefully evaluated, taking into account market milk price evolution, and milking and handling costs.

Udder health has to be continuously monitored by using SCC, conductivity or CMT test. Subclinical mastitis depreciates the value of milk by lowering its quality and severely affects lactation persistency and, lastly, total milk production per ewe.

All the above listed practices aimed to increase lactation persistency should be evaluated taking into consideration their effects on feeding requirements, especially on DM intake in grazing management conditions.

_We would like to acknowledge the technical assistance of Dr. Ana Helena Dias Francesconi._

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