



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).

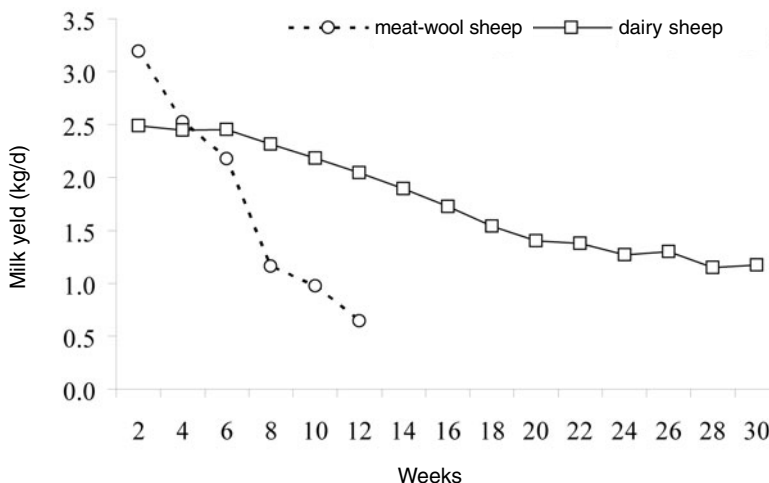
In practice, the ideal lactation curve has a high peak and a moderately flat trend afterwards. More persistent lactation is desirable because it is related to better animal health and reduction of feeding costs (Sölkner and Fuchs, 1987; Pryce *et al.*, 1997; Dekkers *et al.*, 1998; Grossman *et al.*, 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.

Figure 1. Lactation curves of dairy (data from Cappio-Borlino *et al.*, 1997b) and meat-wool (data from Snowder and Glimp, 1991) ewes



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.

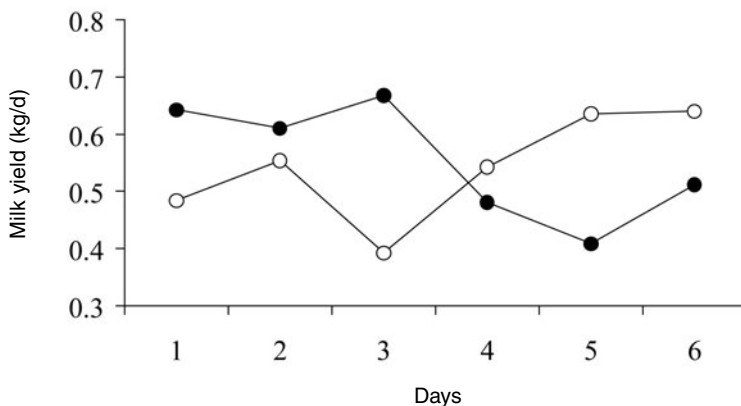
Thus, frequent removal of milk (and consequently of the FIL) from the mammary gland reduces local inhibitory effects on milk synthesis.

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 β -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 α and β 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 (Mephram, 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 (Mephram, 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 Ψ 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

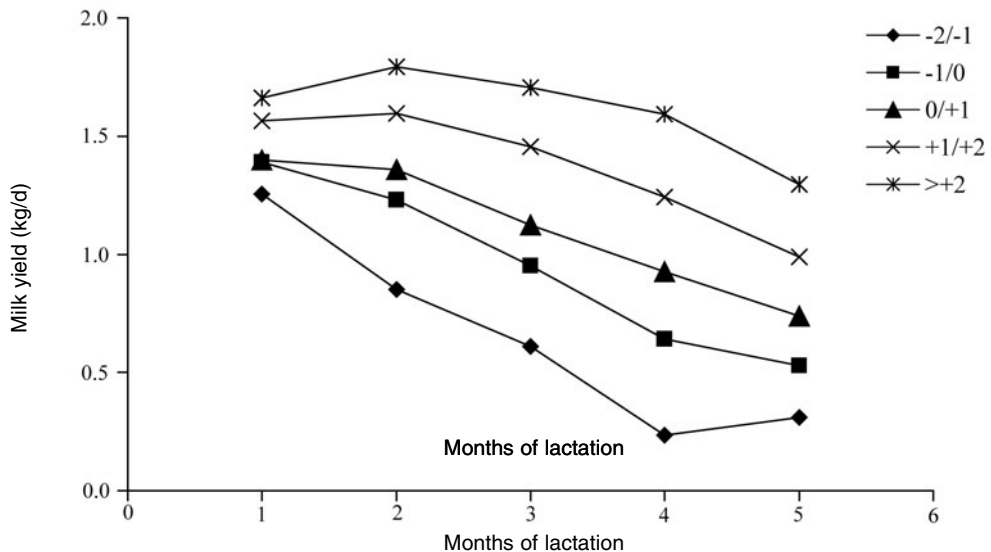
It is well known that exogenous somatotropin (ST) increases milk production in cows and in other dairy ruminants (Akers, 2006). In general, ST administration to dairy ruminants increases milk production in the short term (the immediate post-injection period) and has a medium to long term positive effect 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 1. Correlations between original variables and common factors (Macciotta *et al.*, 2003).

Variable	Milk Yield	
	Factor 1	Factor 2
TD1	0.20	0.85
TD2	0.44	0.82
TD3	0.65	0.53
TD4	0.84	0.30
TD5	0.70	0.19
Variance explained	0.37	0.36

Figure 3. Lactation curves for the milk yield of animals with different classes of factor 1 (Macciotta *et al.*, 2003).



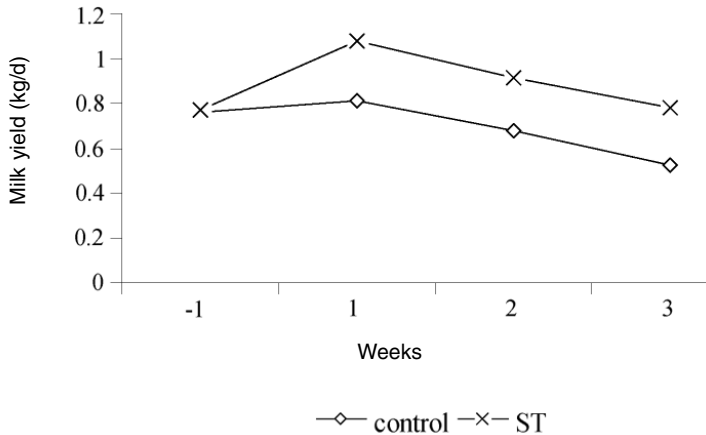
tation (Table 2) or late lactation (Table 3) had milk yields from 20% to 56% higher than controls. Fernandez *et al.* (1997) observed that increasing bovine ST from 160 to 240 mg/head did not increase the milk yield of Manchega dairy ewes. A biological explanation could be a saturation of the hormone effect or a saturation of the mammary storage site between milkings, which leads to an autocrine inhibition of lactation. Fernandez *et al.* (1997) showed that the first part of lactation needs a higher dose of bovine ST than the second one if a maximum increase in milk yield is to be achieved. Primiparous ewes responded better to bovine ST treatment than multiparous ones. In ewes with a body condition score (BCS) ranging from 0 to 5, in which most ewes had a BCS between 3 and 3.5, an interaction between BCS and bovine ST was observed only in the first part of lactation. In that period the highest response to bovine ST was

obtained from ewes with a mean BCS of 3 that received a dose of 200 mg of bovine ST released throughout 14 d. However, BCS had no effect on milk yield during the second part of lactation.

Baldi (1999) reported increases in milk yield by 35%, 27% and 21% in early-, mid- and late-lactation, in dairy ewes treated with bovine ST, without any negative effects on the composition or coagulating properties of milk. In late lactation, bovine ST administration reduced the percentages of fat and protein in milk but improved cheese making efficiency, leading to the production of higher quality cheese.

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).

Figure 4. Milk yield in Comisana ewes treated with exogenous somatotropin (ST) and control in the 3 weeks post-injection (data from D'Urso *et al.*, 1998).



ductive 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

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 repro-

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

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

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

^aoST = ovine somatotropin, bST = bovine somatotropin.

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

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

^abST = bovine somatotropin.

^bLSR = low stocking rate.

^cHSR = high stocking rate.

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 (Frimawayt 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.

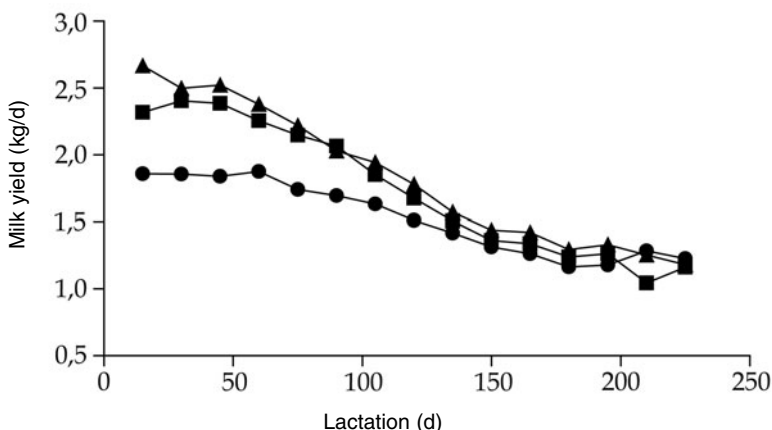
The influence of lambing season on milk yield may also be related to photoperiod. In Mediterranean areas, ewe lactation generally occurs in the period when days are lengthening. In dairy ewes, an increase in the hours of light seemed to increase milk production and feed intake (Bocquier *et al.*, 1997). 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.

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 (Gabiña *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

Figure 5. Lactation curves in ewes of different parity 1 (●), 2 (■), and ≥ 3 (▲); (Cappio-Borlino *et al.*, 1997b).



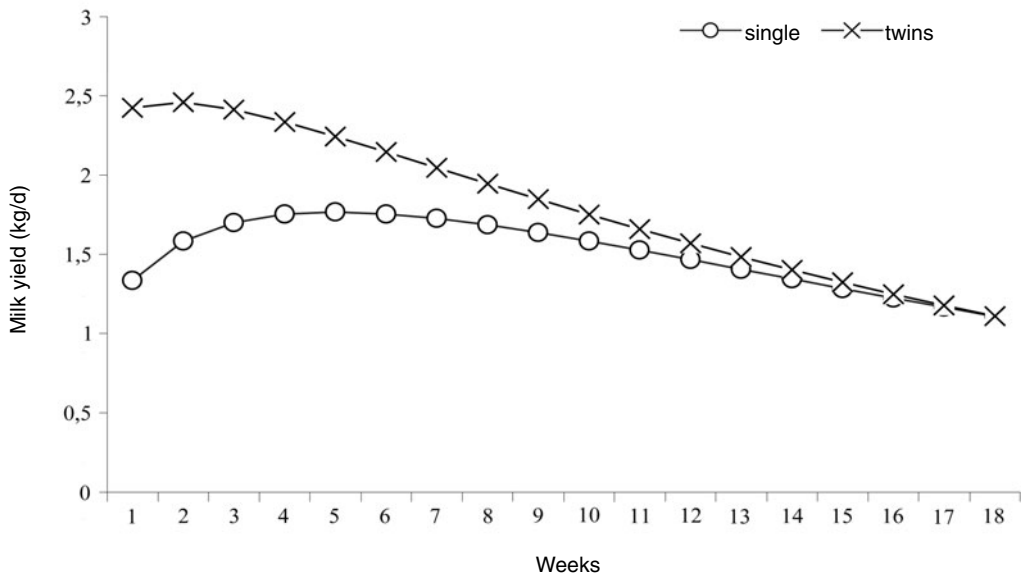
curve becomes similar to that of pluriparous ewes (Cappio-Borlino *et al.*, 1997a; Ruiz *et al.*, 2000). No differences in lactation persistency has been observed in Comisana ewes with different parity (Sevi *et al.*, 2000).

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.

Breed	Purpose	Milk Yield twins vs single	Authors
Delle Langhe	Milk	+10%	Ubertalle <i>et al.</i> , 1990
Sarda	Milk	+11%	Pulina <i>et al.</i> , 1993
Churra	Milk	+4.5%	Gonzalo <i>et al.</i> , 1994
Rambouillet, Columbia, Polypay	Meat-wool	+44%	Snowder and Glimp, 1991
Suffolk	Meat	+63%	Snowder and Glimp, 1991
Merino	Meat-wool	+46-51%	Sokolov and Kuts, 1984

Figure 6. Lactation curves in dairy ewes with different types of lambing (Pulina *et al.*, 1993).



(Butler *et al.*, 1981; Schoknecht *et al.*, 1991). 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⁻¹ 136 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 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

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

Breed	Milk yield kg/d	MY variation in % compared to 2X ^a		Reference
		1X	3X	
Chios	0.891	-21.6	-	Papachristoforou <i>et al.</i> , 1982
Churra	0.803	-47.0	-	Purroy Unanua and Diaz, 1983
Comisana	0.387	-26.4	-	Battaglini <i>et al.</i> , 1979
Comisana	0.440	-21.3	-	De Maria Ghionet <i>et al.</i> , 1982
Lacaune	0.933	-41.0	-	Labussière <i>et al.</i> , 1983
Meat sheep breed	1.430	-20.0	+0.8	Morag, 1968
Poll Dorset	0.494	-7.3	-	Knight and Gosling, 1995
Prealpes du Sud	1.008	-51.3	+14.7	Labussière <i>et al.</i> , 1974
Sarda	1.177	-8.8	-	Enne <i>et al.</i> , 1972
Sarda	1.568	-37.0	+3.0	Cannas <i>et al.</i> , 1991
Tsigai	-	-	+22.6	Gaal, 1958
Tsigai	0.562	-65.0	-	Mykus and Masar, 1978

^a1X, 2X and 3X correspond to once-, twice- and three times daily milking, respectively.

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

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 adrenalin and noradrenalin 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.

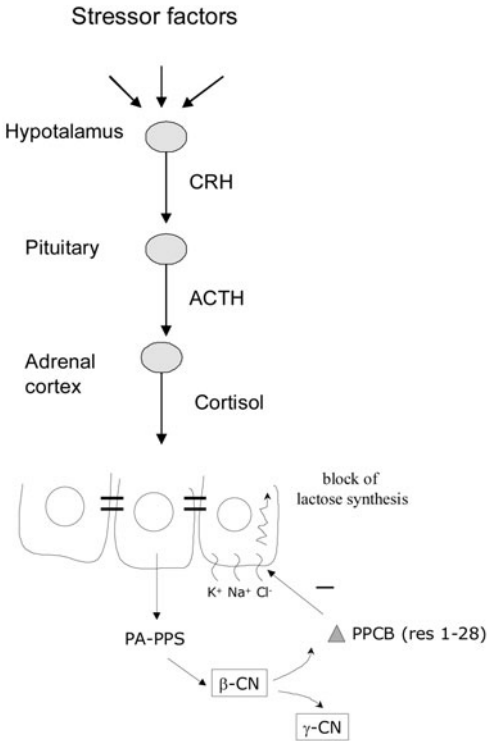
Two main mechanisms may be involved in the response of animal productivity to stress: a local mechanism, proposed by Silanikove *et al.* (2000), which connects the plasmin-plasminogen system to the autocrine inhibition of lactation; and a systemic mechanism which takes

Table 6. Effects of ewes temperament during machine milking on milk production traits (Dimitrov-Ivanov and Djorbineva, 2002).

Functional parameters		Calm n = 106	Nervous n = 54	P
Total morning milk		592	477	**
Machine milk	ml	421	336	*
Machine steaming milk	"	38.3	34.8	ns
Hand steaming milk	ml	137.1	107.4	*
Machine Milking Time	sec	31.4	27.4	*
Milk flow rate	ml/sec	15.6	13.6	*
Milk ejection latency	sec	1.9	5.3	***

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Figure 7. A schematic and simplified representation of the local mechanisms of fraction 1-28 peptide (PPCB) derived from plasmin activity on β -casein into the mammary gland (based on the hypothesis of Silanikove *et al.*, 2000)



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-peptide 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 adrenocorticotrophic 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

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.*).

Leptin class ng/ml	Leptin ng/ml	Milk yield kg	Fat %	FCM ^a kg	DM Intake
<2.30	1.95	1.983	6.90	2.044	2.79
>2.30	2.70	1.434	7.28	1.531	2.22
P	0.000	0.039	0.490	0.034	0.088

^aFCM = Fat corrected milk

in 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*.

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).

Infection status	SCC (x10 ³ /ml)	Milk losses (%)
Uninfected	82	-
Infection by minor pathogens	120	2.6
Unilateral inf. by NSCNS ^a	597	5.1
Unilateral infection by major pathogens	1317	8.8
Bilateral infection by NSCNS	1547	3.6
Bilateral infection by major pathogens	2351	10.1

^aNSCNS = *Novobiocin sensitive coagulase-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.*)

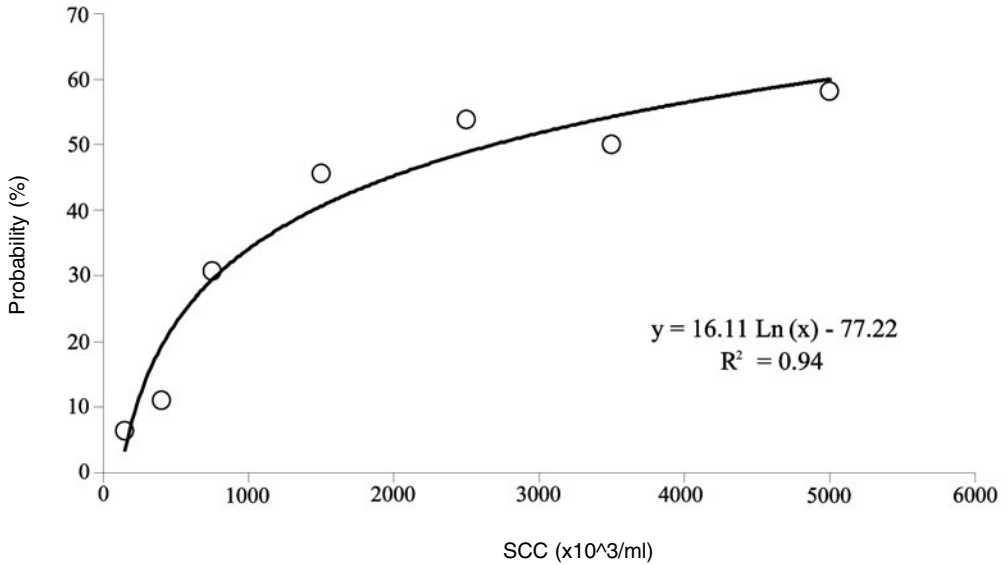


Figure 9. Lactation curves of Sarda dairy ewes positive and negative to the mastitis test (Pulina *et al.*, 1993)

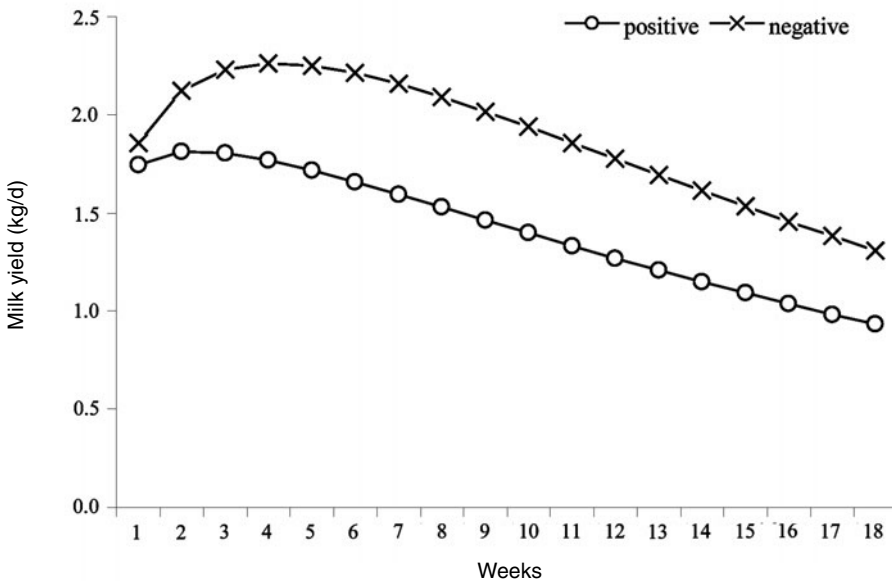


Table 9. Heritability of somatic cell count (SCC) in milk of dairy sheep.

Character	h ²	Breed	Author
LSCS ^a	0.13	Lacaune	Rupp <i>et al.</i> , 2002
LSCS	0.15	Lacaune	Barillet <i>et al.</i> , 2001
Log SCC	0.09	Churra	El-Saied <i>et al.</i> , 1998
Log SCC	0.14	Chios	Ligda <i>et al.</i> , 2002
LSCS	0.12-0.16	Manchega	Serrano <i>et al.</i> , 2003

^aLSCS = Lactation somatic cell score

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).

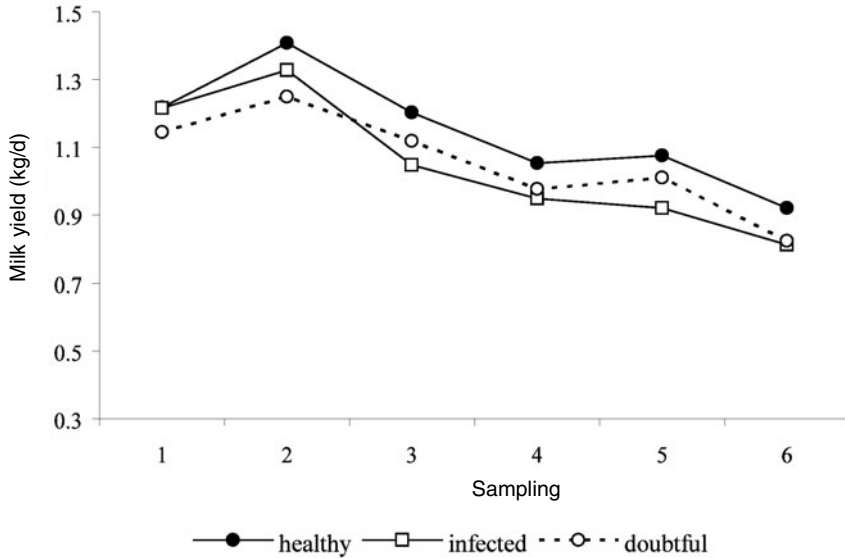
Cows with very low SCC levels may be more susceptible to mastitis than those with higher SCC (Kehrli and Shuster, 1994). Studies based on experimental infection of cows reported that animals resisting udder infection had higher SCC before the pathogenic infection than animals that became infected (Schukken *et al.*, 1998). This may also be true in dairy sheep. In our obser-

vations 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).

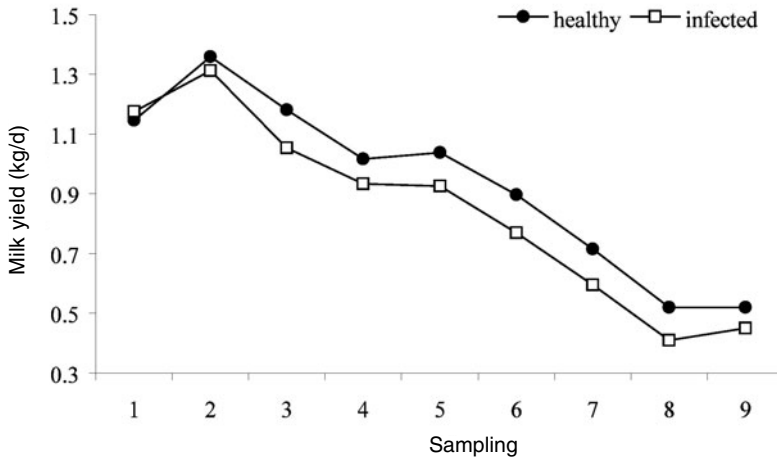
We modified the method of Bergonier and Berthelot (2003) by classifying the ewes in two EUS groups as follows: animals with a SCC under 7.5×10^5 /ml throughout the lactation period were considered healthy (H-ewes), while those with a SCC value above 1×10^6 /ml starting from the second sampling date were 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

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.

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REFERENCES

- ADAMS, N.R., BRIEGEL, J.R., 2005. Multiple effects of an additional growth hormone gene in adult sheep. *J. Anim. Sci.* 83:1868-1874.
- AKERS, R.M., 2002. Lactation and the Mammary Gland. Iowa State Press, Ames, Iowa, USA.
- AKERS, R.M., 2006. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. *J. Dairy Sci.* 89:1222-1234.
- ALBENZIO, M., CAROPRESE, M., SANTILLO, A., MARINO, R., TAIBI, L., SEVI, A., 2004. Effects of somatic cell count and stage of lactation on plasmin activity and cheese-making properties of ewe milk. *J. Dairy Sci.* 87:533-542.
- ALBENZIO, M., TAIBI, L., MUSCIO, A., SEVI, A., 2002. Prevalence and etiology of subclinical mastitis in intensively managed flocks and related changes in the yield and quality of ewe milk. *Small Ruminant Res.* 43:219-226.
- ALLEN, J.C., 1990. Milk synthesis and secretion rates in cows with milk composition changed by oxytocin. *J. Dairy Sci.* 73:975-984.
- BALDI, A., 1999. Manipulation of milk production and quality by use of somatotropin in dairy ruminants other than cow. *Domest. Anim. Endocrinol.* 17:131-137.
- BALDI, A., CHIOFALO, V., SAVOINI, G., GRECO, R., POLIDORI, F., POLITIS, I., 1997. Changes in plasmin, plasminogen and plasminogen activator activities in milk of late lactating ewes: effects of bovine somatotropin (bst) treatment. *Livest. Prod. Sci.* 50:43-44.
- BALDI, A., MODINA, S., CHELI, F., GANDOLFI, F., PINOTTI, L., SCESI, L.B., FANTUZ, F., DELL'ORTO, V., 2002. Bovine somatotropin administration

- to dairy goats in late lactation: effects on mammary gland function, composition and morphology. *J. Dairy Sci.* 85:1093-1102.
- BARILLET, F., 1985. Amélioration génétique de la composition du lait de brebis. L'exemple de la race Lacaune. Ph. D. Degree, Institut National Agronomique, Paris-Grignon, France.
- BARILLET, F., 1997. Genetics in milk production. In: L. Piper and F. Ruwinsky (eds.) *The genetics of sheep*. CAB International, London, UK, pp 539-564.
- BARILLET, F., MARIE, C., JACQUIN, M., LAGRIFFOUL, G., ASTRUC, J.M. 2001. The French Lacaune dairy sheep breed: use in France and abroad in the last 40 years. *Livest Prod. Sci.* 71:17-29.
- BARRY, T.N., 1980. Responses to abomasal infusions of casein plus methionine in lactating ewes fed fresh pasture. *New Zeal. J. Agr. Res.* 23:427-431.
- BATTAGLINI, A., DE MARIA, C., DELL'AQUILA, S., TAIBI, L., 1979. Effetti della soppressione di una mungitura giornaliera sulla produzione e su talune caratteristiche qualitative del latte di pecore di razza Comisana. *Ann. Ist. Sper. Zootec.* 12:1-11.
- BENCINI, R., 1993. The sheep as a dairy animal: lactation, production of milk and its suitability for cheese making. Ph.D. Degree Diss., The University of Western Australia, Perth, Australia.
- BERGONIER, D., BERTHELOT, X., 2003. New advances in epizootiology and control of ewe mastitis. *Livest. Prod. Sci.* 79:1-16.
- BOCQUIER, F., LIGIOS, S., MOLLE, G., CASU, S., 1997. Effect de la photopériode sur la production, la composition du lait et sur les consommations volontaires chez la brebis laitière. *Ann. Zootech.* 46:427-438.
- BORSKI, R.J., 2000. Nongenomic membrane actions of glucocorticoids in vertebrates. *Trends Endocrinol. Metabol.* 10:427-436.
- BRUCKMAIER, R.M., BLUM, J.W., 1998. Oxytocin release and milk removal in ruminants. *J. Dairy Sci.* 81:939-949.
- BURVENICH, C., VANDEPUTTE-VAN MESSOM, G., ROETS, E., MASSART-LEEN, M., PEETERS, G., 1991. Physiological factors affecting milk production in lactating ruminants. pp 1191-1203 in Proc. 9th Nat. Congr. ASPA, Roma, Italy.
- BUTLER, W.R., FULLENKAMP, S.M., CAPIELLO, L.A., HANDWERGER, S., 1981. The relationship between breed and litter size in sheep and maternal serum concentrations of placental lactogen, estradiol and progesterone. *J. Anim. Sci.* 53:1077-1081.
- CANNAS, A., 1996. Nutrition of the dairy ewe. pp 4-21 in Proc. 2nd Great Lakes Dairy Sheep Symp., Madison, WI, USA.
- CANNAS, A., NUDDA, A., PULINA, G., 2002. Nutritional strategies to improve lactation persistency in dairy ewes. pp 17-59 in Proc. 8th Great Lakes Dairy Sheep Symp., Ithaca, NY, USA.
- CANNAS, A., PULINA, G., RASSU, S.P.G., MACCIOTTA, N.P.P., 1991. Influenza della terza mungitura sulla produzione quanti-qualitativa in pecore di razza Sarda. pp 1769-1772 in Proc. 45th Nat. Congr. SISVet, Palermo, Italy.
- CANU, G., DI MAURO, C., NATALE, A., PATTA, C., PULINA, G., 2006. Twin rate influences milk yield in Sarda dairy sheep in organic and conventional farms. *J. Dairy Sci.* 89 (Suppl.1): 57 (abstr).
- CAPPIO-BORLINO, A., MACCIOTTA, N.P.P., PULINA, G., 1997a. The shape of Sarda ewe lactation curve analyzed with a compartmental model. *Livest. Prod. Sci.* 51:89-96.
- CAPPIO-BORLINO, A., PORTOLANO, B., TODARO, M., MACCIOTTA, N.P.P., GIACCONE, P., PULINA, G., 1997b. Lactation curves of Valle del Belice dairy ewes for yields of milk, fat, and protein estimates with Test Day Models. *J. Dairy Sci.* 80:3023-3029.
- CARTA, A., SANNA, S.R., CASU, S., 1995. Estimating lactation curves and seasonal effects for milk, fat, and protein in Sarda dairy sheep with a test day model. *Livest. Prod. Sci.* 44:37-44.
- CASTILLO, V., SUCH, X., CAJA, G., ALBANELL, E., CASALS, R., 2005. Mid-term lactational effects on once versus twice daily milking in Manchega and Lacaune dairy ewes. *J. Dairy Sci.* 88(Suppl.1):W118 (abstr.).
- CASU, S., BOYAZOGLU, J.G., 1974. Effets de la suppression de la traite du soir chez la brebis Sarde. *Ann. Zootech. n. hors-série*:139-144.
- CHANG, Y., REKAYA, R., GIANOLA, D., THOMAS, D.L., 2001. Genetic variation of lactation curves in dairy sheep: a Bayesian analysis of Wood's function. *Livest. Prod. Sci.* 71:241-251.
- CHANG, Y., REKAYA, R., GIANOLA, D., THOMAS, D.L., 2002. Genetic variation of lactation curves in dairy sheep: quadratic function versus Wood's model. *Livest. Prod. Sci.* 77:147-153.
- CHESTNUTT, D.M.B., WYLIE, A.R.G., 1995. The effects of frequency of feeding of supplementary concentrates on performance and metabolite and IGF-I status of ewes given silage in late pregnancy. *Anim. Sci.* 61:269-276.
- CHIOFALO, V., BALDI, A., SAVOINI, G., POLIDORI, F., DELL'ORTO, V., POLITIS, I., 1999. Response of

- dairy ewes in late lactation to recombinant bovine somatotropin. *Small Ruminant Res.* 34:119-125.
- D'URSO, G., AVENDO, M., BORDONARO, S., MARLETTA, D., GUASTELLA, A.M., 1998. Effect of sustained-release somatotropin on performance and grazing behaviour of ewes housed at different stoking rates. *J. Dairy Sci.* 81:958-965.
- DAVIES, S.R., COLLIER, R.J., 1985. Mammary blood flow and regulation of substrate supply or milk synthesis. *J. Dairy Sci.* 68:1041-1058.
- DE BIE, L., BERGER, Y.M., THOMAS, D.L., 2000. The effect of three times a day milking at the beginning of lactation on the milk production of East Friesian crossbreed ewes. pp 1-9. In Proc. 6th Great Lakes Dairy Sheep Symp., Guelph, Ontario, Canada.
- DE MARIA GHIONNA, C., DELL'AQUILA, S., CARINI, S., 1982. Influenza della soppressione di una mungitura giornaliera sulla produzione, su talune caratteristiche qualitative e sull'attitudine alla coagulazione del latte in pecore di razza Comisana. *Zoot. Nutr. Anim.* 8:407-418.
- DEKKERS, J.C.M., TEN HAG, J.H., WEERSINK, A., 1998. Economic aspects of persistency of lactation in dairy cattle. *Livest. Prod. Sci.* 53:237-252.
- DELL'ORTO, V., CHIOFALO, V., SAVOINI, G., ZUMBO, A., SGOIFO ROSSI, C., 1996. Effect de l'injection de somatotropine bovine recombinée (rbST) sur le performances de brebis laitières. Influence de la nature de l'aliment concentré. *Ann. Zootech.* 45:377-383.
- DIMITROV-IVANOV, I., DJORBINEVA, M., 2002. Assessment of welfare, functional parameters of the udder, milk productive and reproductive traits in dairy ewes of different temperament. Page 192 (abstr.) in Proc. 53rd Annual Meet. EAAP, Cairo, Egypt.
- EL SAIED, U.M., CARRIERO, J.A., SAN PRIMITIVO, F., 1998. Heritability of test day somatic cell counts and its relationship with milk yield and protein percentage in dairy ewes. *J. Dairy Sci.* 81:2956-2961.
- ENNE, G., LOSITO DE CARVALHO, R., RALLO, F., 1972. Indagine sulla soppressione delle mungiture domenicali e pomeridiane di pecore di razza Sarda. *Riv. Zoot.* 45:455-472.
- FERNANDEZ, N., RODRIGUEZ, M., PERIS, C., BARCELO, M., MOLINA, M.P., TORRES, A., ADRIAENS, F., 1995. Bovine somatotropin dose titration in lactating dairy ewes. 1. Milk yield and composition. *J. Dairy Sci.* 78:1073-1082.
- FERNANDEZ, N., RODRIGUEZ, M., PERIS, C., MOLINA, M.P., TORRES, A., 1997. Bovine somatotropin dose titration in lactating dairy ewes. 2. Dose determination and factors affecting the response. *J. Dairy Sci.* 80:818-829.
- FLINT, D.J., BOUTINAUD, M., TONNER, E., WILDE, C.J., ACCORSI, P.A., KOLB, A.F., WHITELAW, C.B.A., BEATTIE, J., ALLAN, G.J., 2005. Insulin-like growth factor binding proteins initiate cell death and extracellular matrix remodeling in the mammary gland. *Domest. Anim. Endocr.* 29:274-282.
- FLINT, D.J., KNIGHT, C.H., 1997. Interactions of prolactin and growth hormone (GH) in the regulation of mammary gland function and epithelial cell survival. *J. Mammary Gland Biol. Neoplasia* 2:41-48.
- FRIMAWATY, E., MANALU, W., 1999. Milk yield and lactose synthetase activity in the mammary glands of superovulated ewes. *Small Ruminant Res.* 33:271-278.
- GAAL, M., 1958. Milking Tsigai ewes three times daily at large farms. *Anim. Breeding* 26:236 (abstr.).
- GABIÑA, D., ARRESE, F., ARRANZ, J., BELTRAN DE HEREDIA, I., 1993. Average milk yield and environmental effects on Laxta sheep. *J. Dairy Sci.* 76:1191-1198.
- GENGLER, N., 1996. Persistency of lactation yields: A review. *Interbull Bull.* 12:87-96.
- GONZALO, C., ARIZNABARRETA, A., CARRIEDO, J.A., SAN PRIMITIVO, F., 2002. Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. *J. Dairy Sci.* 85:1460-1467.
- GONZALO, C., CARRIEDO, J.A., BARO, J.A., SAN PRIMITIVO, F., 1994. Factors influencing variation of test day milk yield, somatic cell count, fat, and protein in dairy sheep. *J. Dairy Sci.* 77:1537-1542.
- GROEN, A.F., STEINE, T., COLLEAU, J.J., PEDERSEN, J., PRIBYL, J., REINSCH, N., 1997. Economic values in dairy cattle breeding, with special reference to functional traits. *Livest. Prod. Sci.* 49:1-21.
- GROSSMAN, M., HARTZ, S.M., KOOPS, W.J., 1999. Persistency of lactation yield: a novel approach. *J. Dairy Sci.* 82:2192-2197.
- HENDERSON, A.J., PEAKER, M., 1984. Feed-back control of milk secretion in the goat by a chemical in milk. *J. Physiol.* 351:39-45.
- HOOLEY, R.D., CAMPBELL, J.J., FINDLAY, J.K., 1978. The importance of prolactin for lactation in the ewe. *J. Endocrinol.* 79:301-310.
- HUIDOBRO, F., 1989. Effects of omitting the Sunday afternoon milking on milk yield of Manchega ewes. *Anim. Breeding* 57:4929 (abstr.).
- JAKOBSEN, J., MADSEN, P., JENSEN, J., PEERSEN, J., CRISTIENSEN, L.G., SORESEN, D.A., 2002.

- Genetic parameters for milk production and persistency for Danish Holsteins estimated in random regression models using REML. *J. Dairy Sci.* 85:1607-1616.
- JAMROZIK, J., JENSEN, G., SCHAEFFER, L.R., LIU, Z., 1998. Analysis of persistency of lactation calculated from a random regression test day model. *Interbull Bull.* 16:64-68.
- KEHRLI, M.E., SHUSTER, D.E., 1994. Factors affecting milk somatic cell and their role in health of the bovine mammary gland. *J. Dairy Sci.* 77:619-627.
- KNIGHT, T.W., GOSLING, L.S., 1995. Effects of milking frequency and machine-stripping on the yield and composition of milk from Poll Dorset ewes. *New Zeal. J. Agr. Res.* 38:123-130.
- LABUSSIÈRE, J., COMBAUD, J.F., PETREQUIN, P., 1974. Influence de la fréquence des traites et des tetees sur la production latiere des brebis Prealpes du Sud. *Ann. Zootech.* 23:445-457.
- LABUSSIÈRE, J., DOTCHEWSKI, D., COMBAUD, J.F., 1981. Caractéristiques morphologiques de la mamelle des brebis Lacaune. Méthodologie pour l'obtention des données. Relations avec l'aptitude à la traite. *Ann. Zootech.* 30:115-136.
- LABUSSIÈRE, J., BENNEMEDERBEL, B., COMBAUD, J.F., DE LA CHEVALERIE, F., 1983. Description des principaux parametres caracterisant la production laitiere, la morphologie mammaire et la cinetique d'emission du lait de la brebis Lacaune traitee une ou deux fois par jour avec ou sans egouttage. pp 625-652 in *Proc. 3th Int. Symp. on Machine milking of small ruminant, Valladolid, Spain.*
- LEIBOVICH, H., GERTLER, A., BAZER, F., GOOTWINE, E., 2001. Effect of recombinant placental lactogen and recombinant ovine growth hormone on growth of lambs and milk production of ewes. *Livest. Prod. Sci.* 68:79-86.
- LIGDA, C., MAVROGENIS, A., GEORGIOUDIS, A., 2002. Estimates of genetic parameters for test day somatic cell counts in Chios dairy sheep. pp 9-21 in *Proc 7th WCGALP Congr., Montpellier, France.*
- LUND, T., MIGLIOR, F., DEKKERS, J.C.M., BURNSIDE, E.B., 1994. Genetic relationship between clinical mastitis, somatic cell count, and udder conformation in Danish Holsteins. *Livest. Prod. Sci.* 39:243-251.
- MACCIOTTA, N.P.P., SANNA, S.R., DIMAURO, C., CAPPIO-BORLINO, A., 2003. A multivariate measure of lactation persistency for dairy sheep. *Ital. J. Anim. Sci.* 2(Suppl. 1):40-42.
- MACCIOTTA, N. P. P., VICARIO, D., CAPPIO-BORLINO, A., 2006. Use of multivariate analysis to extract latent variables related to level of production and lactation persistency in dairy cattle. *J. Dairy Sci.* 89:3188-3194.
- MACCIOTTA, N.P.P., VICARIO, D., DI MAURO, C., CAPPIO-BORLINO, A., 2004. A multivariate approach to modeling shapes of individual lactation curves in cattle. *J. Dairy Sci.* 87:1092-1098.
- MANALU, W., SUMARYADI, M.Y., SUDJATMOGO, M.S., SATYANINGTIJAS, A.S., 1998. Effect of superovulation on maternal serum progesterone concentration, uterine and fetal weights at weeks 7 and 15 of pregnancy in Javanese thin-tail ewes. *Small Ruminant Res.* 30:171-176.
- MANALU, W., SUMARYADI, M.Y., SUDJATMOGO, M.S., SATYANINGTIJAS, A.S., 2000. Effect of superovulation prior to mating on milk production performances during lactation in ewes. *J. Dairy Sci.* 83:477-483.
- MATTERI, R.L., CARROLL, J.A., DYER, C.J., 2000. Neuroendocrine responses to stress. In: G.P. Moberg and J.A. Mench (eds.) *The biology of animal stress: Basic principles and implications for animal welfare.* CAB International, Wallingford, UK, pp 43-76.
- MCDUGALL, S., PANKEY, W., DELANEY, C., BARLOW, J., MURDOUGH, P.A., SCRUTON, D., 2002. Prevalence and incidence of subclinical mastitis in goats and dairy ewes in Vermont, USA. *Small Ruminant Res.* 46:115-121.
- MCGUIRE, M.A., BAUMAN, D.E., MILLER, M.A., HARTNELL, G.F., 1992. Response of somatomedins (IGF-I and IGF-II) in lactating cows to variations in dietary energy and protein and treatment with recombinant n-methionyl bovine somatotropin. *Nutr. Rev.* 62:2839 (abstr.).
- MCKUSICK, B.C., BERGER, Y.M., THOMAS, D.L., 1999. Effect of three weaning and rearing systems on commercial milk production and lamb growth. pp 16-31 in *Proc. 5th Great Lakes Dairy Sheep Symp., Battleboro, Vermont, USA.*
- MCKUSICK, B.C., THOMAS, D.L., BERGER, Y.M., 2001. Effect of weaning system on commercial milk production and lamb growth of East Friesian dairy sheep. *J. Dairy Sci.* 84:1660-1668.
- MCKUSICK, B.C., THOMAS, D.L., ROMERO, J.E., MARNET, P.G., 2002. Effect of weaning system on milk composition and distribution of milk fat within the udder of East Friesian dairy ewes. *J. Dairy Sci.* 85:2521-2528.
- MCMURTRY, J.P., MALVEN, P.V., ARAVE, C.W., ERB, R.E., HARRINGTON, R.B., 1975. Environmental and lactational variables affecting prolactin

- concentrations in bovine milk. *J. Dairy Sci.* 58:181-189.
- MEPHAM, T.B., 1987. *Physiology of Lactation*. Milron Keynes, Philadelphia, PA, USA.
- MORAG, M., 1968. The effect of varying the daily milking frequency on the milk yield of the ewe and evidence on the nature of the inhibition of milk ejection by half udder milking. *Ann. Zootech.* 17:351-369.
- MYKUS, M., MASAR, M., 1978. Milk production and labor productivity in sheep milking twice and thrice daily and without stripping. pp 263-276 in *Proc. 2nd Int. Symp. on Machine milking of small ruminant*, Alghero, Italy.
- NOSTRAND, S.D., GALTON, D.M., ERB, H.N., BAUMAN, D.E., 1991. Effects of daily exogenous oxytocin on lactation milk yield and composition. *J. Dairy Sci.* 74:2119-2127.
- NEGRÃO, J.A., MARNET, P.G., 2003. Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. *Small Ruminant Res.* 47:69-75.
- NUDDA, A., BENCINI, R., MIJATOVIC, S., PULINA, G., 2002a. The yield and composition of milk in Sarda, Awassi and Merino sheep milked unilaterally at different frequencies. *J. Dairy Sci.* 85:2879-2885.
- NUDDA, A., FELIGINI, M., BATTACONE, G., MURGIA, P., PULINA, G., 2001. Relationship between somatic cell count, whey protein and coagulation properties in sheep milk. pp 511-513 in *Proc. 14th Nat. Congr. ASPA*, Firenze, Italy.
- NUDDA, A., PULINA, G., BATTACONE, G., CAPPIOBORLINO, A., BRANDANO, P., 2002b. Use of ultrasound technique for measuring mammary cistern size in primiparous and secondiparous dairy ewes. Page 220 (abstr.) in *Proc. 53rd Annual Meet. EAAP*, Cairo, Egypt.
- NUDDA, A., PULINA, G., VALLEBELLA, R., BENCINI, R., ENNE, G., 2000. Ultrasound technique for measuring mammary cistern size of dairy ewes. *J. Dairy Res.* 67:101-106.
- PADAYATTY, S.J., ORME, S., ZENOBI, P.D., STICKLAND, M.H., BELCHETZ, P.E., GRANT, P.J., 1993. The effects of insulin-like growth factor-1 on plasminogen activator inhibitor-1 synthesis and secretion: results from in vitro and in vivo studies. *Thromb. Haemost.* 70:1009-1013.
- PAPACHRISTOFOROU, C., ROUSHIAS, A., MAVROGENIS, A.P., 1982. The effect of milking frequency on the milk production of Chios ewes and Damascus goats. *Ann. Zootech.* 31:37-46.
- PLAUT, K., BAUMAN, D.E., AGERGAARD, N., AKERS, R.M., 1987. Effect of exogenous prolactin administration on lactational performance of dairy cows. *Domest. Anim. Endocrinol.* 4:279-290.
- POLITIS, I., BLOCK, E., TURNER, J.D., 1990. Effect of somatotropin on the plasminogen and plasmin system in the mammary gland: proposed mechanism of action for somatotropin on the mammary gland. *J. Dairy Sci.* 73:1494-1499.
- POLITIS, I., 1996. Plasminogen activator system: implications for mammary cell growth and involution. *J. Dairy Sci.* 79:1097-1107.
- PORTOLANO, B., TODARO, M., SPATAFORA, F., BONO, G., MARGOTTA, S., GIACCONE, P., ORTOLEVA, V., 1996. Confronto fra due differenti modelli della curva di lattazione in pecore da latte di razza Comisana. *Zoot. Nutr. Anim.* 22:323-331.
- PRYCE, J.E., VEERKAMP, R.F., THOMPSON, R., HILL, W.G., SIMM, G., 1997. Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. *Anim. Sci.* 65:353-360.
- PULINA, G., NUDDA, A., 1996. Aspetti tecnici e fisiologici della mungitura meccanica degli ovini. *Inform. Agr.* 41:69-74.
- PULINA, G., RASSU, S.P.G., CANNAS, A., 1993. The effect of group feeding strategy on milk production in dairy ewes. pp 2003-2006 in *Proc. 47th Nat. Congr. SISVet*, Riccione, Italy.
- PULINA, G., RASSU, S.P.G., NUDDA, A., BATTACONE, G., 2002. Influenza del fotoperiodo e dell'intermungitura sulla produzione di latte in pecore sarde. Page 230 in *Proc. 15th Nat. Congr. SIPAOC*, Chia (CA), Italy.
- PULINA, G., NUDDA, A., FANCELLU, S., BARBATO, A.M., RUBATTU R., 2005. Infusion of casein hydrolizates into mammary gland simulates the omission of one daily milking in goats. *Ital. J. Anim. Sci.* 4(suppl. 2):386-388.
- PURROY UNANUA, A., DIAZ, M.J.L., 1983. Características de ordino de raza Churra: producción y composición de leche; morfología de la ubre y cinética de emisión de leche. pp 984-993 in *Proc. 3rd Int. Symp. on Machine milking of small ruminant*, Valladolid, Spain.
- RASSU, S.P.G., CANNAS, E.A., NICOLUSSI, P., BONELLI, P., PULINA, G., 2006. The impact of machine milking on milk production traits and cortisol in primiparous dairy ewes. *J. Dairy Sci.* 89(suppl. 1):303 (abstr.).
- REKAYA, R., WEIGEL, K.A., GIANOLA, D., 2001. Hierarchical nonlinear model for the persistency of milk yield in the first three lactations of Holsteins. *Livest. Prod. Sci.* 68:181-187.
- ROVAL, M., SUCH, X., CAJA, G., PIEDRAFITA, J., 2002. Changes in cisternal and alveolar milk throughout lactation in dairy sheep. *J. Dairy Sci.*

- 85(suppl. 1):12(abstr.).
- RUIU, A., PULINA, G., 1992. Mastiti ovine, qualità del latte e prospettive di profilassi sanitaria. *Inform. Agr.* 14:37-40.
- RUIZ, R., OREGUI, L.M., HERRERO, M., 2000. Comparison of models for describing the lactation curve of Latxa sheep and an analysis of factors affecting milk yield. *J. Dairy Sci.* 83:2709-2719.
- RUPP, R., BOICHARD, D., BARBAT, A., ASTRUC, J.M., LAGRIFFOUL, G., BARILLET, F., 2002. Selection for mastitis resistance in French dairy sheep. pp 9-28 in *Proc. 7th WCGALP Congr., Montpellier, France.*
- RUSHEN, J., DE PASSILLE, A.M., MUNKSGAARD, L., 1999. Fear of people by cows and effects on milk yield, behavior, and heart rate at milking. *J. Dairy Sci.* 82:720-727.
- SCHOKNECHT, P.A., NOBREGA, S.N., PETTERSON, J.A., EHRHARDT, R.A., SLEPETIS, R., BELL, A.W., 1991. Relations between maternal and fetal plasma concentrations of placental lactogen and placental and fetal weights in well-fed ewes. *J. Anim. Sci.* 69:1059-1063.
- SCHUKKEN, Y.H., LESLIE, K.E., BARNUM, D.A., MALLARD, B.A., LUMSDEN, J.H., DICK, P.C., VESSIE, G.H., KEHRLI, M.E., 1998. Experimental *Staphylococcus aureus* intramammary challenge in late lactation dairy cows: quarter and cow effects determining the probability of infection. *J. Dairy Sci.* 82:2393-2401.
- SERRANO, M., PEREZ-GUZMAN, M.D., MONTORO, V., JURADO, J.J., 2003. Genetic analysis of somatic cell count and milk traits in Manchega ewes. Mean lactation and test-day approaches. *Livest. Prod. Sci.* 84:1-10.
- SEVI, A., TAIBI, L., ALBENZIO, M., MUSCIO, A., ANNICCHIARICO, G., 2000. Effect of parity on milk yield, composition, somatic cell count, renneting parameters and bacteria counts of Comisana ewes. *Small Ruminant Res.* 37:99-107.
- SHAMAY, A., SHAPIRO, F., MABJEESH, S.J., SILANIKOVE, N., 2002. Casein-derived phosphopeptides disrupt tight junction integrity, and precipitously dry up milk secretion in goats. *Life Sci.* 70:2707-2719.
- SILANIKOVE, N., SHAMAY, A., SHINDER, D., MORAN, A., 2000. Stress down regulates milk yield in cows by plasmin induced β -casein product that blocks k^+ channels on the apical membranes. *Life Sci.* 67:2201-2212.
- SILVA, L.P.T., VANDERHARR, M.S., WEBER NIELSEN, M.S., ETCHEBARNE, B.E., 2002. Leptin reduces proliferation of a bovine mammary epithelial cell line. *J. Dairy Sci.* 85(suppl. 1):338.
- SNOWDER, G.D., GLIMP, H.A., 1991. Influence of breed, number of suckling lambs, and stage of lactation on ewe milk production and lamb growth under range conditions. *J. Anim. Sci.* 69:923-930.
- SOKOLOV, V.V., KUTS, G.A., 1984. Sheep's milk - a valuable food product. *Anim. Breeding* 52:517 (abstr.).
- SÖLKNER, J., FUCHS, W., 1987. A comparison of different measures of persistency with special respect to variation of test day yields. *Livest. Prod. Sci.* 16:305-319.
- STANTON, T.L., JONES, L.R., EVERETT, R.W., KACHMAN, S.D., 1992. Estimating milk, fat and protein lactation curves with a test day model. *J. Dairy Sci.* 75:1691-1700.
- STELWAGEN, K., KNIGHT, C.H., 1997. Effect of unilateral once or twice daily milking of cows on milk yield and udder characteristics in early and late lactation. *J. Dairy Res.* 64:487-494.
- STELWAGEN, K., GRIEVE, D.G., WALTON, J.S., BALL, J.L., MCBRIDE, B.W., 1993. Effect of prepartum bovine somatotropin in primigravid ewes on mammogenesis, milk production, and hormone concentrations. *J. Dairy Sci.* 76:992-1001.
- STELWAGEN, K., VAN ESPEN, D.C., VERKERK, G.A., MCFADDEN, H.A., FARR, V.C., 1998. Elevated plasma cortisol reduces permeability of mammary tight junctions in the lactating bovine mammary epithelium. *J. Endocrinol.* 159:173-178.
- STELWAGEN, K., DAVIS, S.R., FARR, V.C., PROSSER, C.G., SHERLOCK, R.A., 1994. Mammary epithelial cell tight junction integrity and mammary blood flow during an extended milking interval in goats. *J. Dairy Sci.* 77:426-432.
- SWALVE, H.H., 1995. Genetic relationship between dairy lactation persistency and yield. *J. Anim. Breed. Genet.* 112:303-311.
- SWALVE, H.H., 2000. Theoretical basis and computational methods for different test day genetic evaluation methods. *J. Dairy Sci.* 83:1115-1124.
- THOMAS, D.L., BERGER, Y.M., MCKUSICK, B.C., 2001. Effects of breed, management system, and nutrition on milk yield and milk composition of dairy sheep. *J. Anim. Sci.* 79(suppl. E):16-20.
- THORN, S.R., PURUP, S., COHICK, W.S., VESTERGAARD, M., SERJSEN, K., BOISCLAIR, Y.R., 2006. Leptin does not act directly on mammary epithelial cells in prepuberal dairy heifers. *J. Dairy Sci.* 89:1467-1477.
- TOGASHI, K., LIN, C.Y., 2003. Modifying the lactation curve to improve lactation milk and persistency. *J. Dairy Sci.* 86:1487-1493.

- TONNER, E., ALLAN, G.J., FLINT, D.J., 2000. Hormonal control of plasmin and tissue-type plasminogen activator activity in rat milk during involution of the mammary gland. *J. Endocrinol.* 167:265-273.
- UBERTALLE, A., BIANCHI, M., ERRANTE, J., BATTAGLINI, L.M., 1990. Prolificità e produzione latte: correlazioni fenotipiche in pecore Delle Langhe. *Zoot. Nutr. Anim.* 16:219-224.
- WILDE, C.J., HENDERSON, A.J., KNIGHT, C.H., BLATCHFORD, D.R., FAULKNER, A., VERNON, R.G., 1987. Effects of long-term thrice-daily milking on mammary enzyme activity, cell population and milk yield in the goat. *J. Anim. Sci.* 64:533-539.
- WOOD, P.D.P., 1967. Algebraic model of the lactation curve in cattle. *Nature* 216:164-165.
- ZAMIRI, M.J., QOTBI, A., IZADIFARD, J., 2001. Effect of daily oxytocin injection on milk yield and lactation length in sheep. *Small Ruminant Res.* 40:179-185.