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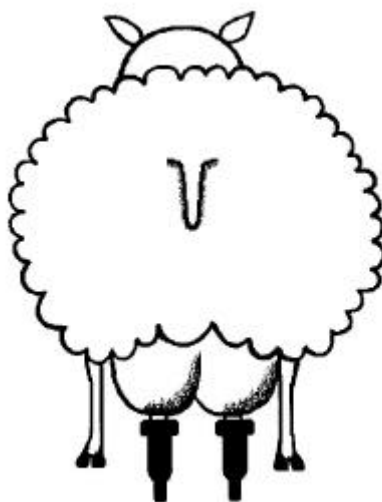
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# NON-NUTRITIONAL STRATEGIES TO IMPROVE LACTATION PERSISTENCY IN DAIRY EWES

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## Introduction

Milk production is largely dependent on the shape of the lactation curve. Important elements in the lactation pattern are the peak yield, which is the maximum milk yield during lactation, and lactation persistency, which is the ability of animals to maintain a reasonably constant milk yield after the lactation peak. “Persistent” animals are those with flatter lactation curves.

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

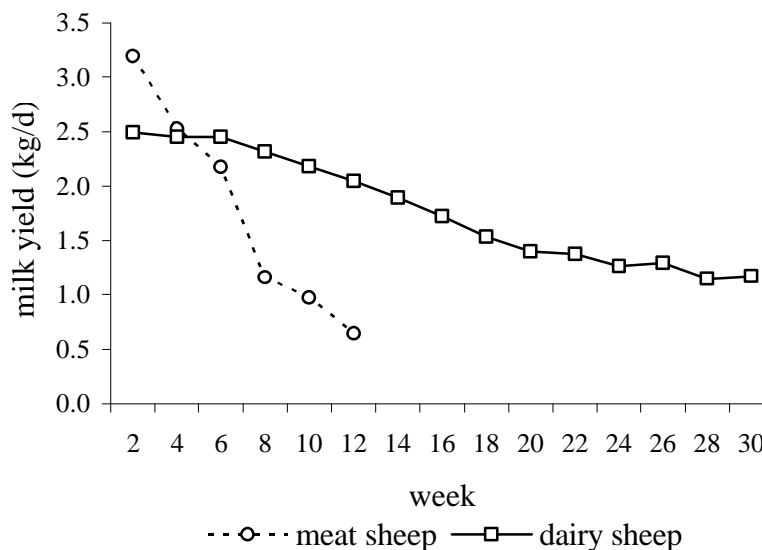


Figure 1 - Lactation curves of dairy (data from Cappio-Borlino et al., 1997b) and meat-wool sheep (data from Snowder and Glimp, 1991).

In dairy sheep, genetic selection has caused deep morphological changes in the udder and physiological changes in the whole body of the animal. The former are seen in the higher mammary cistern volume and the latter in neuro-hormonal changes that allow the alveoli to have a longer life-span and maintain a metabolic status that favors the switch of energy and nutrients to the mammary gland instead of body reserves. In practice, the ideal lactation curve has a reasonably high peak and a flat trend after the peak. More persistent lactation is desirable due to the relationships between this trait and health status and feed costs (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 a negative energy balance, reduced reproductive efficiency and increased susceptibility to diseases (Jakobsen et al., 2002; Swalve, 2000). By contrast, animals with flat curves are less subject to metabolic stress in early lactation and have a more constant pattern of energy requirements throughout lactation. This means that cheaper feeds can be used (Sölkner and Fuchs, 1987; Dekkers et al., 1998).

In most cases the milk of the first month of lactation is suckled by the lamb. This means that there is less milk yield data available on the ascending phase of lactation, which consequently has been little studied.

### **The Economic Impact of Lactation Persistency**

The lactation curves of sheep have certain peculiarities. These are due to biological and, above all, management factors. In Mediterranean countries the reproductive and productive cycles are strictly seasonal and are synchronized with the availability of natural pasture: the two periods of grass growth are autumn and spring. Feed supplements are given only in some periods of the year: hay from late summer to autumn and concentrates from late autumn to winter. This means that milk production is strongly influenced by environmental factors (Macciotta et al., 1999). As a result, different types of lactation curves can be observed in the same area within the same breed. For instance, one can often observe curves which are smooth in the first part of lactation (with no lactation peak) due to adverse environmental conditions (such as low temperatures and scarce feed availability) and curves that present a “false” lactation peak in the second half of lactation due both to favorable climatic conditions in spring and, more importantly, the greater availability of pasture (Cappio-Borlino et al., 1997a). Pulina et al. (2001) developed a static and deterministic bio-economic model for these types of breeding systems. This model included many biological and economic factors and was used to calculate the economic values of milk production and feed intake in dairy sheep farms. The model was implemented in the OVISOFT2® software (Boe and Pulina, 2005) which was tested in several dairy sheep farms in Italy with good results in various combinations of management conditions. OVISOFT2® simulates the daily milk yield by using the Wood’s lactation curve  $y = at^b \exp^{-ct}$  (Wood, 1967) where the parameter  $a$  is related to initial milk production,  $b$  represents the slope of the curve in the ascendant phase and  $c$  indicates the slope of the curve in the descending phase. Dairy ewes may differ in total milk yield because of differences in persistency but have the same peak yield or, contrariwise, have different peak yields but similar lactation persistency. Two simulations were carried out using Ovisoft® in a standard flock of 100 Sarda ewes (average BW 45 kg and total milk yield (TMY) 280 kg per ewe lambing in Nov), in order to evaluate the economic impact of changes in lactation persistency. It was assumed that the total milk yield and lactation length was the same in the two simulations. In other words, the values for parameters  $a$  and  $c$  in Wood’s lactation curve equation varied (Table 1). All other inputs (biological, technical and economic variables) remained constant in both simulations.

Table 1 – Values of parameters *a*, *b* and *c* for the simulations of low (L-pers) and high (H-pers) persistency of lactation.

		Month of lambing						
		Nov	Dec	Jan	Feb	Mar	Apr	May
L-pers	a	1232.23	1232.23	1232.23	1232.23	1232.23	1232.23	1232.23
	b	0.23	0.23	0.20	0.18	0.27	0.29	0.23
	c	-0.045	-0.045	-0.05	-0.055	-0.062	-0.065	-0.045
H-pers	a	990.52	990.52	990.52	990.52	990.52	990.52	990.52
	b	0.23	0.23	0.20	0.18	0.27	0.29	0.23
	c	-0.0315	-0.0315	-0.035	-0.0385	-0.0434	-0.0455	-0.0315

Under these conditions the economic impact of an increase in lactation persistency (H-persistency), simulated by reducing the absolute value of *c* parameter by 30% compared to the low persistency curve (L-persistency), was evaluated. The curve of each flock is the mean of the lactation curves of animals with delivery distributed in different months of the year (Figure 2).

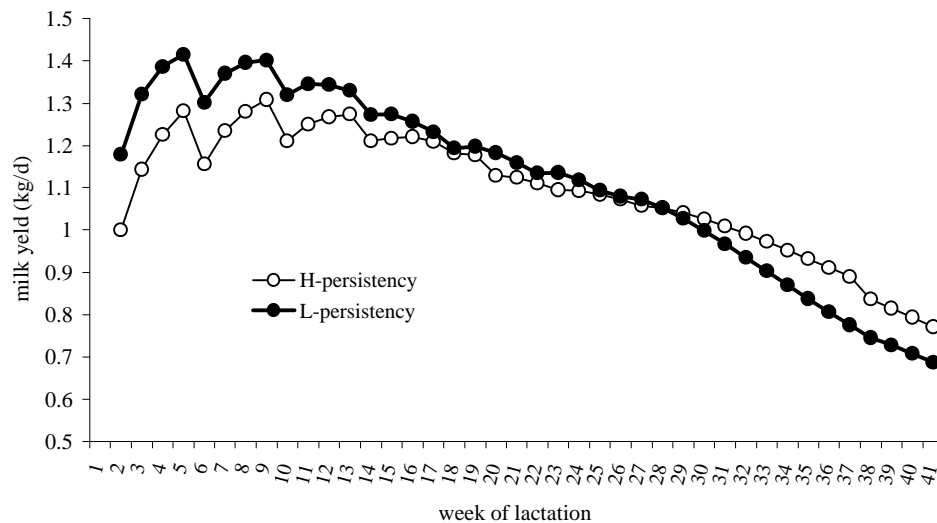


Figure 2 - Lactation curves of ewe flocks using an Ovisoft® software (Boe and Pulina, 2005) simulation. The values for parameters *a* and *c* of Wood’s equation varied while all the remaining inputs, including lactation length and total milk yield, remained fixed. The curve for each flock is the mean of the lactation curves of animals with delivery distributed over different months of the year.

Analysis of the economic output of the simulation showed that an increase in lactation persistency reduced farm operating costs by 2% and gave an annual added value per animal of \$1.00. The outputs of these simulations showed that dairy sheep farms are more profitable if the flock’s lactation persistency can be increased, even when there is no increase in milk production

## **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 area 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. Knowledge of the physiological and environmental factors that influence the number and the activity of mammary secretory cells is needed in order to develop a proper strategy for maintaining lactation. Maintenance of milk synthesis and secretion is controlled by a combination of both systemic and local regulatory factors.

### **Systemic factors**

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

During lactation, GH (Akers, 2002) and PRL (McMurtry et al., 1975) levels decrease. This reduces milk synthesis. GH receptors are not present in the mammary gland. It exerts its positive effects on milk yield indirectly by stimulating the synthesis and secretion of insulin-like growth factor-I (IGF-I). IGF-I is mainly synthesized by the liver, but it is produced and acts in other tissues also, such as, for example, the mammary parenchyma. IGF-I receptors have been identified in the mammary glands of sheep (Akers, 2002). GH administration increases IGF-I in serum, which means that GH may help the mammary epithelial cells to survive. IGF-I is, indeed, a stimulatory protein in DNA synthesis and in mammary proliferation, in casein gene expression, and in glucose transport. Secretion of IGF-I is regulated by the nutritional status of animals. For example, plasma IGF-I concentration increases when high-energy and high-protein diets are used (McGuire et al., 1992). Increasing the frequency of feeding with concentrates from one to three times a day, or improving the quality of forage, increases IGF-I plasma concentrations in ewes in late pregnancy (Chestnutt and Wylie, 1995). GH treatments may be a useful 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 IGFBP-5 (IGF binding protein), which antagonizes the effects of IGF-I on the survival of mammary epithelial cells (Tonner et al., 2000). A reduction in serum PRL concentration reduces the milk yield and results in a 20-25% loss in the number of secretory cells within 48 h (Flint and Knight, 1997). In sheep if bromocriptine, an alkaloid that inhibits the release of PRL, is administered 10 days after parturition, there is a 60-70% reduction in milk production (Burvenich et al., 1991).

## 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 on milk yield and the negative effects of milk stasis in the mammary cistern. The accumulation of milk in the mammary gland accelerates the involution process and reduces lactation 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 milk yield without effecting the milk yield of the other udder, which continued to be milked twice a day (Figure 3).

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 by the mammary epithelial cells and secreted with the milk in the alveoli. As time from last 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.

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).

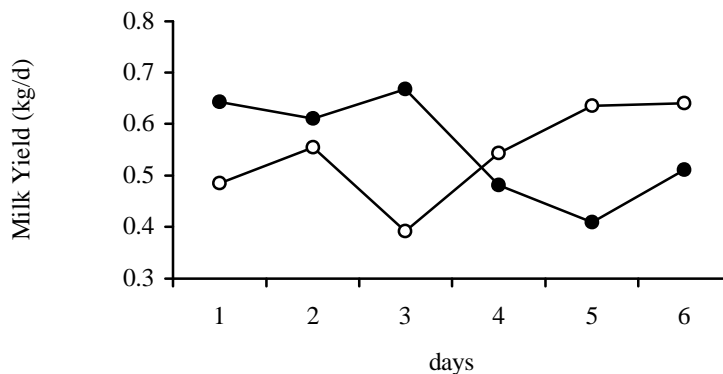


Figure 3 - Milk production (g/d) of right (●) and left (○) udder halves milked once or twice a day. The right udder halves were milked twice a day (2X) for the first period of the experiment and once a day (1X) in the second period. The left udder halves were milked once a day in the first period and twice a day in the second period (Nudda et al., 2002a).

Recently, 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 cows and goats. 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 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.

### **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 plasminogen 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 plasminogen and plasmin increases in milk as lactation progresses.

The plasma insulin-like growth factor (IGF-I), which acts as a mediator of the growth hormone (GH), and the 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 in 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 tight junctions (TJ) between adjacent cells. The TJ are 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 permit the passage between cells of blood precursors that 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 (see review of Cannas et al., 2002).

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).

Tight junctions can be damaged by: the increased activity of plasmin, as lactation progresses, in the 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 the tissue 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).

It appears, then, that mammary involution is controlled by local and systemic factors with highly integrated mechanisms of control.

### **Non-Nutritional Factors Affecting Lactation Persistency**

A proper definition of strategies to improve lactation persistency requires knowledge of the several factors that affect lactation persistency. There include genetics, hormonal status, seasonal effects, management techniques, animal health (e.g. mastitis) and stress.

The influence of feeding on lactation persistency in dairy ewes was reviewed previously (Cannas et al., 2002), while factors other than nutrition are discussed more deeply in this paper.

### **Genetics**

The genetic modification of the shape of the lactation curve in an economically desirable direction is an interesting challenge for scientists and technicians in the dairy industry (Rekaya et al., 2001). Several studies have been carried out in dairy cattle on the relationships between fundamental traits of lactation curve shape such as persistency and peak yield, and productive and functional traits. The favorable relationships which exist between persistency and feeding costs, metabolic status, and disease resistance have been highlighted in dairy cows (Dekkers et al., 1998; Sölkner and Fuchs, 1987; Pryce et al., 1997). However, the strategies to genetically improve this trait are less defined and clear. At present, the main constraint is the lack of consensus on the most suitable measure of persistency. Several approaches have been proposed in the literature (Gengler, 1996; Grossman et al., 1999; Jamrozik et al., 1998; Sölkner and Fuchs, 1987; Togashi and Lin, 2003). These 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. One result of this variety of approaches is the wide range of estimated values for genetic parameters of lactation persistency that are found in the literature, depending on the measure used to define this trait. To take just one example, heritability goes from a value of around zero to values higher than 0.30. The relationship between persistency and total lactation yield is another issue. Some measures of persistency show a high correlation with accumulated milk yield, even though some authors state that a robust measure should be independent of 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), and that selection for this trait is feasible.

Genetic aspects of lactation curve shape have been little investigated in dairy sheep. At present in this species, the main breeding goal is cumulated 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 TD records available. In the typical dairy sheep farming system of Mediterranean countries where most of dairy sheep flocks are located, the milk of the first month of lactation is suckled by the lamb and thus data for this period (which is when the lactation peak occurs) are not available. However when one considers that the dairy sheep farming system has 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, for example, lactation curve shape traits, could be of great value.

The genetic variation of features of lactation curve shape in sheep has been investigated by Chang et al. (2001, 2002), using a quadratic function and the Wood's model. 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 selecting 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 time distances from parturition are considered different traits and are analysed with the multivariate Factor Analysis technique.

In the Factor approach, the correlation matrix of original variables ( $\mathbf{S}$ ) is decomposed as

$$\mathbf{S} = \mathbf{B}\mathbf{B}' + \mathbf{Y}$$

where  $\mathbf{B}$  is the matrix of the factor coefficients, i.e. of the correlations between the new latent variables and the original variables, and  $\mathbf{Y}$  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. By 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  $\mathbf{B}$  matrix obtained by applying Factor Analysis on milk test day (TD) records of 380 Sarda breed dairy ewes is shown in Table 2. Each ewe had 5 TD records, and these were considered to be different traits.

Table 2 - Correlations between original variables and common factors.

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

Two common factors were able to explain about 73% of the original variance. Factor 1 is associated with the TD of the last part of lactation and can be considered to be an indicator of lactation persistency, whereas Factor 2 is correlated with the tests of the first part, and can be considered to be an indicator of production levels in early lactation. The relationships between Factor 1 scores and lactation curve shape can be inferred from Figure 4 where the average lactation patterns of five different classes of animals, grouped according to Factor 1 scores, are shown. One can see that as the value of Factor 1 increases, the persistency of lactation tends to increase. A mixed model analysis of the 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.

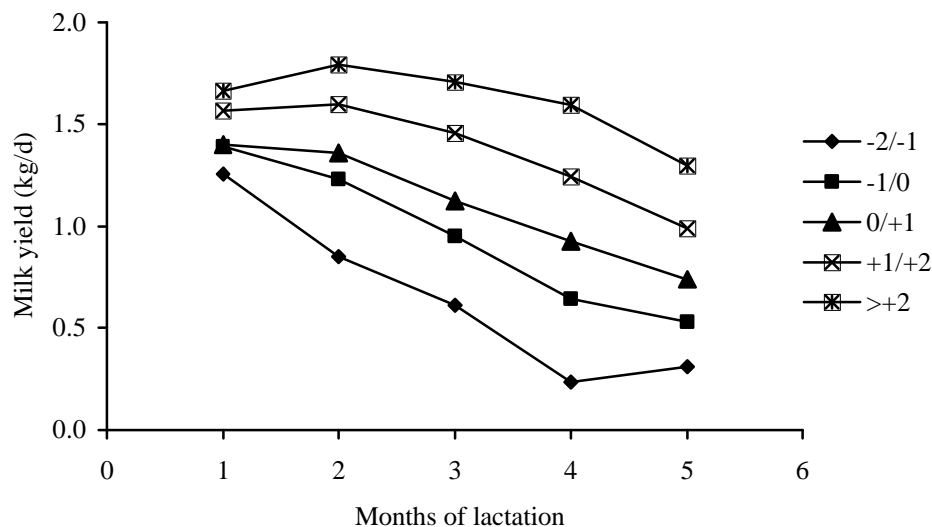


Figure 4 - Lactation curves for the milk yield of animals with different classes of factor 1.

### Use of hormones

It is well established that exogenous somatotropin (ST) increases milk production in cows and in other dairy ruminants. ST increases the concentration of somatomedins (IGF) in the blood. These are involved in the mechanism by which exogenous ST treatments increase milk production in middle and late lactation. In general ST administration studies on dairy ruminants

show that milk production increases in the short term (the immediate post-injection period) and that there is also a medium to long term positive effect on lactation persistency (Baldi 1999). The administration of 320 mg of ST to Comisana ewes increased the milk yield significantly (Figure 5) (D'Urso et al., 1998).

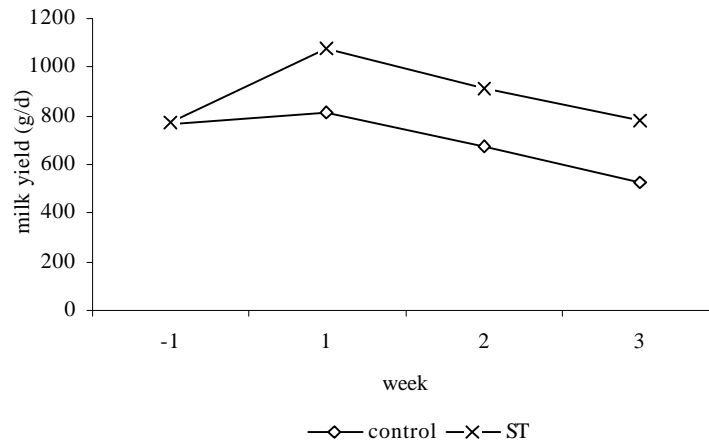


Figure 5 - Milk yield in Comisana ewes treated with exogenous somatotropin (ST) and control in the 3 weeks post-injection (data from D'Urso et al., 1998).

Several studies indicate that ST administration increases milk yield by 10-40% in cows (Flint et al., 2005) and by 14–29% in dairy goats (Baldi, 1999).

Baldi (1999) reported an increased milk yield in dairy ewes treated with ST, without any negative effects on the composition or coagulating properties of the milk, except in late lactation. In that period ST reduced the percentages of fat and protein in milk, although the coagulation time was lower in treated animals.

In other trials, dairy ewes treated with ST during pregnancy, in early-mid lactation (Table 4) and in late lactation (Table 5), had milk yields from 20% to 56% higher than controls. Fernandez et al. (1997) observed that increasing ST from 160 to 240 mg/head did not increase the milk yield of Manchega dairy ewes. A biological explanation could be that there was a plateau phase caused by the saturation of the effect of the hormone or by the 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 ST than the second if a maximum increase in milk yield is to be achieved. The number of lactations did not improve milk production, but primiparous ewes responded better than multiparous ones to ST treatment. An interaction between body condition score and ST was observed only in the first part of lactation. This was when the highest response to ST was obtained from ewes with average body condition score 3 that received a dose of 200 mg of ST released throughout 14 d. However the body condition score had no effect on milk yield during the second part of lactation.

Table 4 - Response of dairy ewes to somatotropin (ST) administration in early-mid lactation.

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

Table 5 - Response of dairy ewes to somatotropin (ST) administration in late lactation.

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

The role of ST in sheep has also been recently investigated by the production of ST transgenic sheep with doubled levels of ST 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 Brigel, 2005).

The daily injection of oxytocin (2 IU) in Mehraban ewes 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% greater for the oxytocin treated group than for the control group (Figure 6). In this study the parameters of the lactation curve were not estimated. However in a similar experiment in dairy cows (Nostrand et al., 1991), it was observed that the oxytocin group produced 849 kg more milk during lactation than the control group, with a significant difference occurring after peak milk yield and greater persistency of lactation.

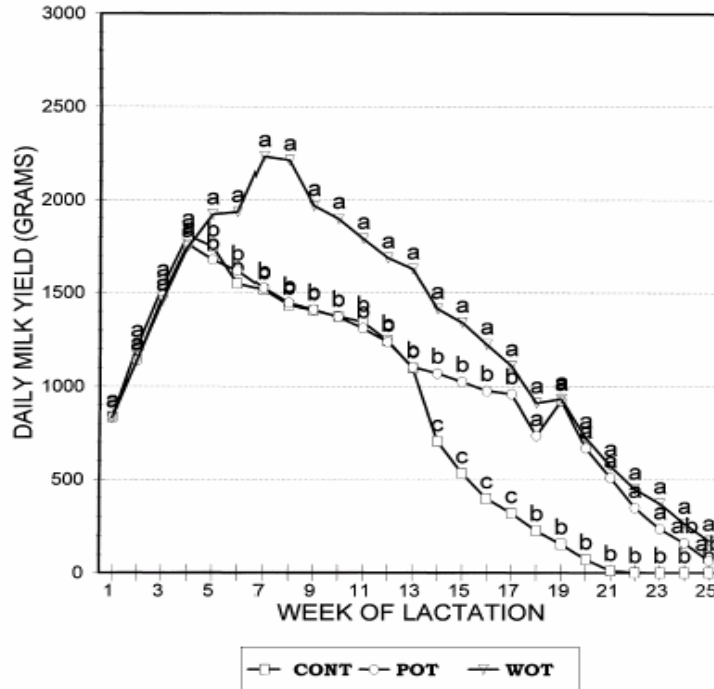


Figure 6 - Lactation curves of ewes given daily oxytocin injection over the whole lactation (WOT) or during the post-weaning period (POT) compared to saline treated control ewes (CONT) (Zamiri et al., 2001).

### Lambing season

The effects of the 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). The ewes that lambed when the maximum amount of forage was available had a higher milk yield, perhaps because of a positive effect on the differentiation of udder secretory cells and on the accumulation of body reserves.

The influence of the lambing season on milk yield may also be related to the photoperiod. In Mediterranean areas, lactation occurs during the period when the days are lengthening. As has also been observed in dairy cows, the increase in the hours of light seems to improve 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 the animals feed more when there is more light. Indeed, sheep which were submitted, for a short period, to sharp changes in day length produced less milk (Pulina et al., 2002).

### Lactation number

Analysis of the evolution of the shape of the lactation curve according to the number of lambings showed that Laxta (Gabina et al., 1993), Lacaune (Barillet, 1985), 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. By contrast, the peak yield took place quite late in 1<sup>st</sup> lactation sheep and lactation is more persistent in 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, the effect of the fact that the animal is still maturing is evident only in the first part of lactation (70-120 days in milking (DIM)), after which it gradually becomes less pronounced and the rest of the 1<sup>st</sup> lactation curve becomes similar to that of pluriparous ewes (Cappio-Borlino et al., 1997a; Ruiz et al., 2000). Portolano et al. (1996) observed that there was an interaction between the effects of the lambing season and the lactation number on the shape of the lactation curve in Comisana dairy ewes. Ewes which lambed in autumn showed greater persistence, smaller peak production and reached this peak later than the same parity ewes which lambed in winter. This phenomenon may be due to the environmental and nutritional effects of different lambing seasons on grazing management conditions. In fact, the peak milk production for ewes lambing in autumn is depressed by the effects of winter, and they can only take advantage of more and better quality pasture after the lactation peak.

### Type of lambing

Several studies have reported higher milk yields for ewes with multiple births, in both non-dairy (Figure 7) (Wohlt et al., 1984) and dairy sheep (Figure 8) (Pulina et al., 1993). This can be explained by the fact that ewes rearing multiple fetuses or with a heavier single fetus have higher placental weight and higher serum progesterone and more placental lactogen hormones during pregnancy (Butler et al., 1981; Schoknecht et al., 1991). The higher average serum progesterone levels during pregnancy means that mammary glands are better developed at parturition as can be seen from the greater number of mammary cells and the increased synthetic activity (Manalu et al., 1998; 2000). In addition, because the mammary glands are suckled more frequently by twins than by one lamb, the local inhibitors to milk secretion, such as the FIL, are removed.

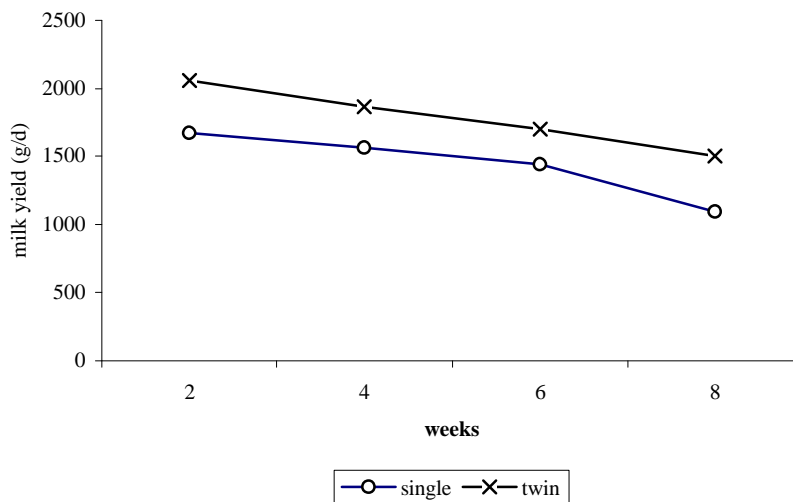


Figure 7 - Lactation curves in Dorset ewes with different types of lambing (Wohlt et al., 1984).

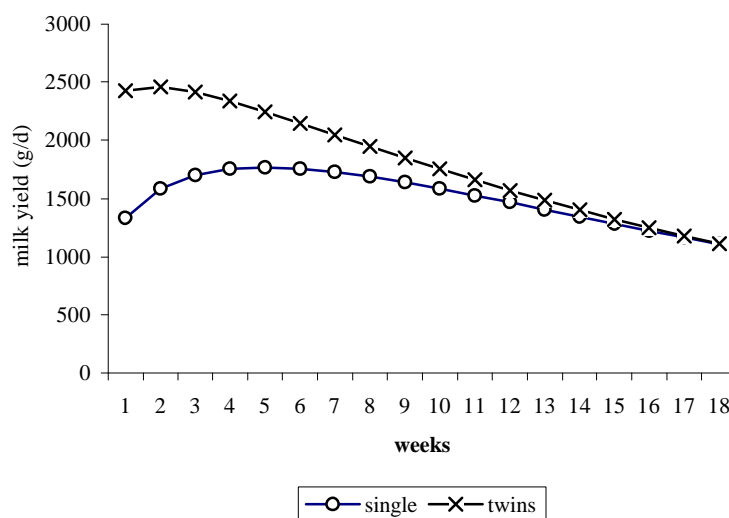


Figure 8 - Lactation curves in dairy ewes with different types of lambing (Pulina et al., 1993).

Ewes superovulated with Pregnant mare serum gonadotropin (PMSG), were found to have 31% better developed mammary glands at parturition and 55% greater milk production during the first 12 weeks of lactation (Frimawaty and Manalu, 1999). This is because superovulation prior to mating increases the numbers of corpora lutea and mean serum progesterone concentrations during pregnancy (Manalu et al., 1998). However, Frimawaty and Manalu (1999) did not observe differences in milk yield between ewes rearing single or twin lambs. Analysis of the mammary glands at the end of lactation indicated that superovulated ewes had 79% higher total DNA and 56% higher total RNA than non-superovulated ewes (Manalu et al., 2000). This indicates that there were more secretory cells and higher synthetic activity per cell.

### 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 saleable milk. However particular attention has to be paid to the weaning technique used, because it could reduce milk yield after weaning.

Studies on different weaning systems were carried out during the first 30 days of lactation. In these studies, ewes were either: milked twice daily after weaning at 24 hours post partum (D1), suckled lambs for 30 days and were 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 the ewes to be machine milked once daily the following morning (MIX) (McKusick et al., 1999; 2001). Total commercial milk production in the MIX ewes was only 10% lower than D1 (236 vs. 261 kg) and 37% higher than the D30 ewes (172 kg). Average lactation length (suckling + milking period) was similar in the various weaning systems. McKusick et al. (2002) compared the MIX and D1 weaning systems in East Friesian crossbreed ewes and found a higher milk yield in MIX ewes in weeks 2 and 4 postpartum. This was probably due to more frequent and

complete udder evacuation by the suckling lambs than by machine milking, as the latter reduces local concentrations of a feedback inhibitor of lactation.

In another study on East Friesian ewes, Thomas et al. (2001) observed that raising lambs on milk replacer and starting the milking of ewes 24–36 hours after parturition increased milk production by 61% when 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 and reduce lactation persistency through a mechanism that involves systemic and local factors, as described previously in this paper.

In dairy sheep, once per day milking (1X) reduced milk yield when compared to twice daily milking (2X) with similar intensity in dairy and non-dairy sheep breeds (Table 6) (Pulina and Nudda, 1996).

Table 6 - Influence of milking frequency on milk yield (MY) in dairy and non-dairy sheep

Breed	Milk yield, Kg/d	MY variation in % compared to 2X		Reference
		1X	3X	
Chios	0.891	-21.6	-	Papachristoforou et al., 1982
Churra	0.803	-47.0	-	Purroy Unanua and Diaz, 1983
Comisana	0.387	-26.4	-	Battaglini et al., 1979
Comisana	0.440	-21.3	-	De Maria et al., 1982
Lacaune	0.933	-41.0	-	Labussière et al., 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 et al., 1974
Sarda	1.177	-8.8	-	Enne et al., 1972
Sarda	1.568	-37.0	+2.0	Cannas et al., 1991
Tsigai	-	-	+22.6	Gaal, 1958
Tsigai	0.562	-65.0	-	Mykus and Masar, 1989

When, however, the ewes are milked more than twice per day, then the effect on non-dairy ewes is greater than in dairy ewes (Bencini, 1993). For example, increasing milking frequency from twice to three times per day only increased milk yield for the whole lactation period by 3% in Sarda ewes (Cannas et al 1991), while in Merino ewes it increased milk yield by about 21% (Bencini, 1993). The difference is probably due to the smaller udder storage capacity of Merino ewes compared to Sarda ewes. If the udder capacity is low then the milk must be removed more frequently. In an experiment with East Friesian crossbreed ewes, the responses to an increase in milking frequency in the first 30 days of lactation was related to the genetic potential of the animals (de Bie et al., 2000). In this trial, 25% of the animals did not show any response to a third milking, 50% of ewes produced 13% more milk, and 37.5% of the animals produced 36% more milk during the first 30 days of lactation. Probably, 37.5% of the ewes had



a genetic potential to produce more milk but had limited udder storage capacity. If this is the case then the more often the udder is milked, the more milk the ewe can produce. It is worth pointing out that when the third milking was removed, the milk yield dropped immediately to the level of twice a day milking (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.

A reduction in milk yield has also been reported when one evening milking per week was removed. The reduction varied with the breed, from 7.0% in Poll Dorset (Knight and Gosling; 1995) to 8.5% in Manchega (Huidobro, 1989), 14% in Sardinian (Casu and Boyazoglu, 1974) and 25.6% in Prealpes du Sud (Labussière et al., 1974). The magnitude of the effect of missing 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 breed (-33%) than in the Lacaune breed (-10%). The authors attributed the result to the lower cistern storage capacity of Manchega ewes (63%) compared to Lacaune ewes (77%), and the way that this can increase the negative effects of local factors on milk secretion.

### **Udder morphology and cistern dimension**

As 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 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 (Bencini, 1993; Labussière et al., 1981) and cistern dimension (Nudda et al., 2000; Rovai et al., 2002). The use of ultrasound techniques to measure cistern size found that there was 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 that the action of the FIL should be less in animals with larger cisterns was tested in an experiment in which dairy and non-dairy breeds were compared (Nudda et al., 2002a). We observed that two breeds, which were highly selected for milk production (Sarda and Awassi), responded to the reduction in the frequency of milking from twice to once a day by producing 18% to 24% less milk. Similar results were seen in Merino ewes, a wool breed not selected for milk production (Nudda et al., 2002a). This result is probably due to the fact that while the cisterns of the Merinos were smaller so was their average yield, and so the ratios between milk volume and milk cistern storage capacity were similar in dairy and non-dairy breeds. In the same trial it was also observed that the reduction of milk yield with once per day milking increased in proportion with the production level of the Sarda ewes, while in Merino ewes the reduction was independent of the production level. This was probably because the latter produced very little milk.

## Stress

Reducing the emotional or physical stress of dairy animals will help to increase their productivity and maintain their health status. The effects of human contact (Rushen et al., 2001), a gentle or rough handler during milking (Munksgaard et al., 2001), and the use of the preferred side of the milking parlour (Paranhos da Costa and Broom, 2001) on milk yield have been analyzed in dairy cows. Dimitrov-Ivanov and Djorbineva (2002) found that machine-milked calm ewes produced more milk than nervous ones (Table 7). Agitation and excitement in the milking parlour is probably influenced by both genetic factors and the previous handling experience of the animals. In cattle it has been observed that animals with previous experience of quiet handling will be calmer and easier to handle in the future. In dairy cows the presence of a rough handler did not modify the total milk yield per milking but increased the residual milk by 70% (Rushen et al., 1999), which affected the milking length.

Breuer et al. (2000) carried out a survey on 31 farms and observed that several variables related to rough stockperson behaviour were negatively correlated with cow productivity. To be precise, they found that the behaviour used when forcing cows into position in the milking shed and/or when moving cows out of the shed were significantly correlated with milk yield ( $r=-0.40$  and  $-0.39$ , respectively,  $P<0.05$ ). They also found negative correlations between the number of loud or harsh sounds used by the stockperson and milk yield, and protein and fat content.

Table 7 -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
<i>Total morning milk</i>	ml	592	477	**
Machine milk	ml	421	336	*
Machine steaming milk	ml	38.3	34.8	NS
<i>Hand steaming milk</i>				
	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	***

The speed of movement of the stockperson when moving the cows from pasture to the milking shed over the last 50 m was also negatively correlated with milk yield. Thus it seems that fear of humans may have practical implications for the productivity of commercial dairy animals, including sheep. Our preliminary results showed that primiparous ewes that started to enter the milking parlor 2 weeks before weaning the lambs were calmer than ewes that entered the milking parlor after weaning. There was also higher milk yield and lower milk SCC at the beginning of lactation (Rassu et al., unpublished data).

Others physical stress such as water deprivation (Senn et al., 1996) or high temperatures, can negatively influence milk production. Restricting water consumption to 50% of the voluntary water intake for four days decreased the milk yield in cows by 74%

when compared with the control group. In addition, the restricted cows behaved very aggressively around their water trough and spent more time in its vicinity (Little et al., 1980). Whether the animals return to their normal productive capacity after the stress depends on the lactation stage, and in fact decreases as lactation progresses. 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 plasminogen-plasmin system to the autocrine inhibition of lactation; a systemic mechanism which takes 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 plasmin activator (PA) from the mammary epithelial cells into the mammary cistern where it activates the plasmin system that degrades  $\beta$ -casein and produces the residue 1-28  $\beta$ -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 9). When injecting the 1-28  $\beta$ -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.

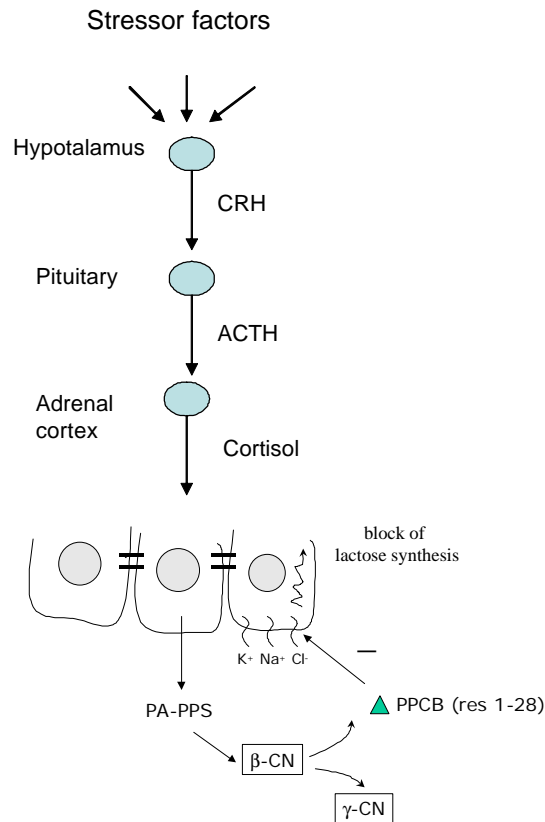


Figure 9 - A schematic and simplified representation of the local mechanisms of fraction 1-28 peptide (PPCB) derived from plasmin activity on  $\beta$ -casein into the mammary gland.

In the systemic mechanism, stress activates the HPA axis: the response to different stress factors provokes firstly the release of the hypothalamic factor 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 shows itself in 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 resulted in the cortisol concentrations being substantially higher and a reduction of mammary tight junction leakiness (Stelwagen et al., 1998), which showed itself in involution of the mammary gland (see paragraph 2). A secondary effect of stress is the inhibition of prolactin synthesis by the pituitary gland, due to the hypothalamic release of dopamine. Both cases lead the lactating ewe to a transient metabolic energy unbalance, due to the reduction in the energy output by the milk and an increase in mobilization of stored energy. This is caused by a sharp increase in glucocorticoids, followed by an increase in insulin and adipose tissue uptake capacity. If the stress level remains, it may have a negative effect on lactation persistency, especially in the second half of lactation, due to the leptin hormone secreted by adipose tissue inhibiting the 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 8).

Table 8 - Relationship between leptin, milk yield and DM intake in Sardinian breed ewes (Cannas et al., unpublished data).

Leptin class ng/ml	Leptin ng/ml	Milk yield kg	Fat %	FCM <sup>a</sup> 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

<sup>a</sup>FCM = Fat corrected milk

### Udder health status

Although clinical cases of mastitis are a source of milk loss, subclinical mastitis is more important economically because it occurs more frequently (Ruiu and Pulina, 1992). It is associated with a decrease in milk production, milk quality and coagulation properties (Nudda et al., 2001). The coagulase negative staphylococci (CNS) 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 a higher somatic cell count (SSC) in milk (Figure 10) (Pulina et al., unpublished data).

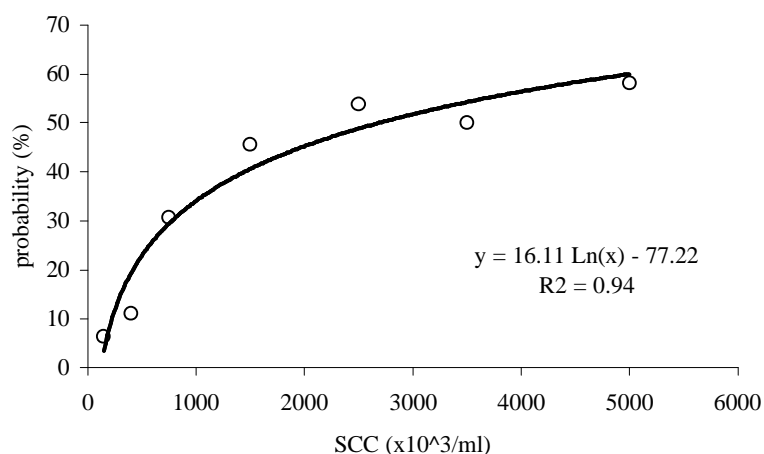


Figure 10 – Relationship between the probability of isolating microorganisms and somatic cell count (SCC) in half udders of dairy sheep (Pulina et al., unpublished data).

Losses of milk yield through intramammary infection (IMI) in sheep varies with the type of pathogen. High SCC, corresponding to major pathogens, causes larger milk yield losses (Table 9) (Gonzalo et al., 2002).

Table 9 - 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 <sup>3</sup> /ml)	Milk losses (%)
Uninfected	82	-
Infection by minor pathogens	120	2.6
Unilateral inf. by NSCNS <sup>a</sup>	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

<sup>a</sup>NSCNS = Novobiocin sensitive coagulase-negative staphylococci

Sarda breed ewes with mammary glands, which were positive on bacteriological analysis, suffered a reduction in milk yield of about 24% in overall lactation when compared to negative animals (Figure 11). The pattern showed that intramammary infections (IMI) before the peak caused a reduction in peak yield, and the milk yield loss is maintained during lactation with a consequent lower persistency (Pulina et al., 1993).

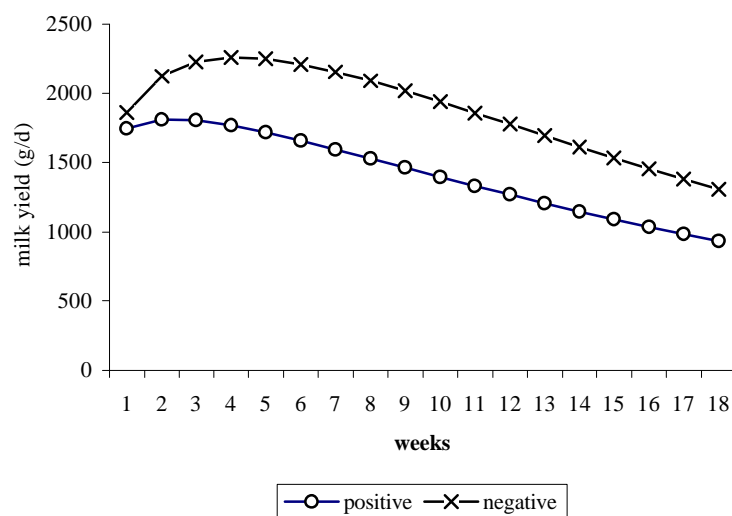


Figure 11 – Lactation curves of dairy ewes positive and negative to the mastitis test (Pulina et al., 1993)

The fight against mastitis in small ruminants is necessary not only because of the economic losses in milk yield and penalties in the payments of milk with high SCC, but also because it is necessary to improve the health status and welfare of animals.

Direct selection for mastitis resistance has been considered inefficient because SCC heritability, as an indirect measurement of udder health, is low in dairy sheep (Table 10), as it is also in dairy cows (Lund et al., 1994).

Table 10 - Heritability of somatic cell count (SCC) in milk of dairy sheep

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

<sup>a</sup>LSCS = Lactation somatic cell count

Some studies have indicated that cows with very low SCC levels may be more susceptible to mastitis than cows 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, we discovered that all the 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 a greater number of samples.

Bergonier and Berthelot (2003) proposed a method for estimating the presence of subclinical mastitis. 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” in other cases. Using this method on the 90 Sarda ewes in our dataset with 6 samplings of each ewe, we classified three estimated udder health status (EUS) groups. The EUS classes significantly influenced both milk yield (Figure 12) and composition. The infection of the mammary gland was accompanied by a marked decrease in lactose, and a significant increase in the whey protein derived from blood (serum albumin, lactoferrin and immunoglobulin).

In Figure 13 we show the lactation curves of two groups of Sarda ewes that at the beginning of lactation were homogeneous for milk yield and SCC in milk ( $< 5 \times 10^5$ /ml). In our method, animals were classified in two EUS groups as follows: animals with a SCC under  $7.5 \times 10^5$ /ml throughout the lactation period were considered healthy (H-ewes), while those with a SCC value above  $1 \times 10^6$ /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 the rate of decline after the peak (i.e. persistency), but was mainly due to the rapid loss of efficiency of synthesis of their secretory cells.

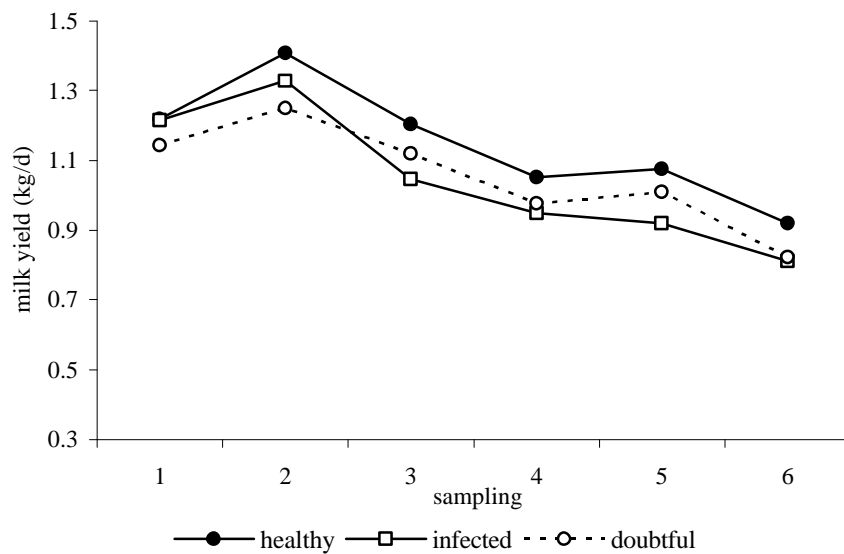


Figure 12 - Milk lactation curve in Sarda dairy ewes with udder classified healthy, doubtful and infected using the method of Bergonier and Berthelot (2003).

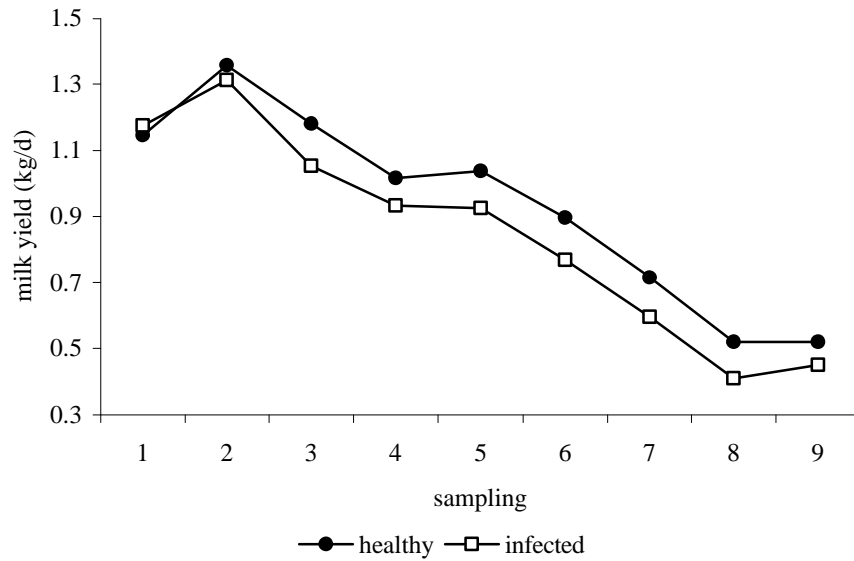


Figure 13 - Milk lactation curve in Sarda dairy ewes with udder classified healthy and infected using our method.

### Practical Implications

The economic importance of persistency makes it desirable to have a flock with many ewes with lactation curves as flat as possible.

Even though exogenous administration of hormones (like ST, PRL and OT) effectively increases milk yield, we believe that permanent and profitable results can be achieved by: a) enhancing technical practices, b) focusing on better genetic goals for dairy sheep, 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 the 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 conveniently be adopted for sheep 1-80 days in milking (DIM), 80-160 DIM and >160 DIM, respectively. However, milk yield gains/losses over the continuous 2X routine must be carefully evaluated, taking into account market milk price evolution, milking and handling costs and the interference with feeding requirements, and especially the DM intake in grazing management conditions.



Finally, 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.

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