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# NUTRITIONAL STRATEGIES TO IMPROVE LACTATION PERSISTENCY IN DAIRY EWES

**Antonello Cannas, Anna Nudda, Giuseppe Pulina**

**Dipartimento di Scienze Zootecniche  
University of Sassari  
Sardinia, Italy**

## INTRODUCTION

Milk production is largely dependent on the shape of the lactation curve. Relevant elements of the lactation pattern are the peak yield, which represents the maximum milk yield during the lactation, and the lactation persistency, which expresses the ability of animals to maintain a reasonably constant milk yield after the lactation peak (Figure 1). Thus, persistent animals are those that show flatter lactation curves. Several measurements of persistency have been proposed (Broster and Broster, 1984; Gengler, 1996): the rate of fall of milk yield per week or per month; combinations of parameters of mathematical functions used to model the lactation curve; the variation of test day yields throughout the whole lactation or part of it; the proportion of total milk yield achieved in a certain period (e.g. second half of lactation). However, none of the above mentioned measurements seems to be able to become the reference method (Grossman et al., 1999). For example, the definition of persistency as the rate of fall of milk yield per unit of time can be misleading if the absolute level of production is not considered. Usually curves with high peak yield show low persistency because the rate of milk yield declines faster in animals that have a fast milk yield increase after calving (Figure 1). Thus in this review, we will consider persistency in a broad sense, and we will analyze the nutritional and non-nutritional factors that affect and limit milk production in mid-late lactation in sheep.

In dairy cows the correlation between peak yield and lactation yield (+.78) was higher than that observed between persistency and lactation yield (+.34) (Galton and Merril, 2002). In dairy ewes as in cows, milk yield in the first month of lactation, i.e. when the lactation peak is attained, was highly correlated with total milk production per lactation (Rossi, 1976). Thus peak yield seems to be more important in determining the total lactation yield than persistency even though, as already said before, these results should be further confirmed by comparing individual shapes of the lactation curve within production levels. Nevertheless, more persistent lactation may be desirable due to the relationships between this trait and reproduction efficiency, health status and feed costs (Dekkers et al. 1998; Grossman et al., 1999). For example, animals with very high peak yield are not able to consume adequate amounts of nutrients in the first part of lactation. This results in 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 subjected to metabolic stress in early lactation and have a more constant pattern of energy requirements throughout the lactation, allowing the utilization of cheaper feeds (Solkner and Fuchs, 1987; Dekkers et al., 1998).

Very long lactations are not desirable either, because a non-lactating (dry) period prior to parturition is required to restore mammary gland secretory tissue for milk production in the subsequent lactation. In general a period of 50 days of dry period is recommended in cows, even though in goats the omission of the dry period between lactations did not reduce subsequent milk production (Fowler

et al., 1991). In sheep very long lactations are rare and in general the dry period is sufficiently long to restore mammary gland secretory tissue.

Lactation curves of sheep show several peculiarities that are the result of biological and, above all, management factors (Figure 2; Cappio-Borlino et al., 2002). In Mediterranean countries, where there is the world's largest concentration of dairy sheep, reproductive and productive cycles are strictly seasonal, being synchronized with the availability of natural pasture; as a consequence, milk production is strongly influenced by environmental factors (Macciotta et al., 1999). Moreover, in most cases the milk of the first month of lactation is suckled by the lamb. This has reduced the availability of milk yield data for the ascending phase of lactation, which has been little studied.

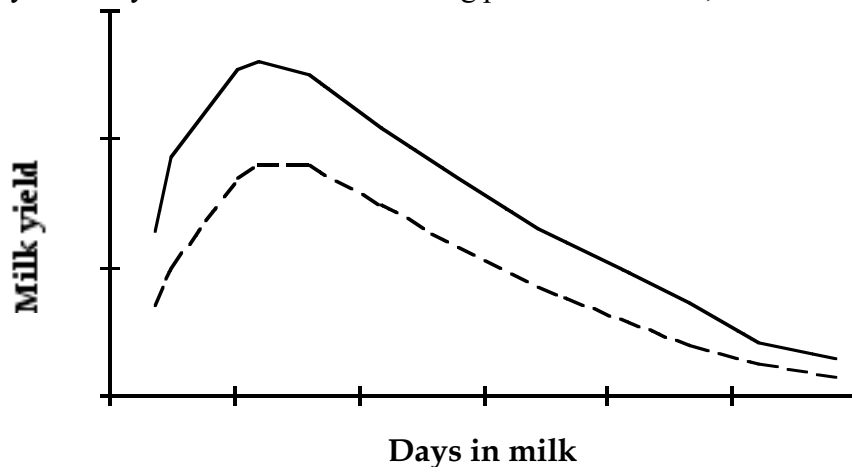


Figure 1 - Typical lactation curves of ruminants. The higher curve (continuous line) has higher peak milk yield and lower persistency than the second (dotted line).

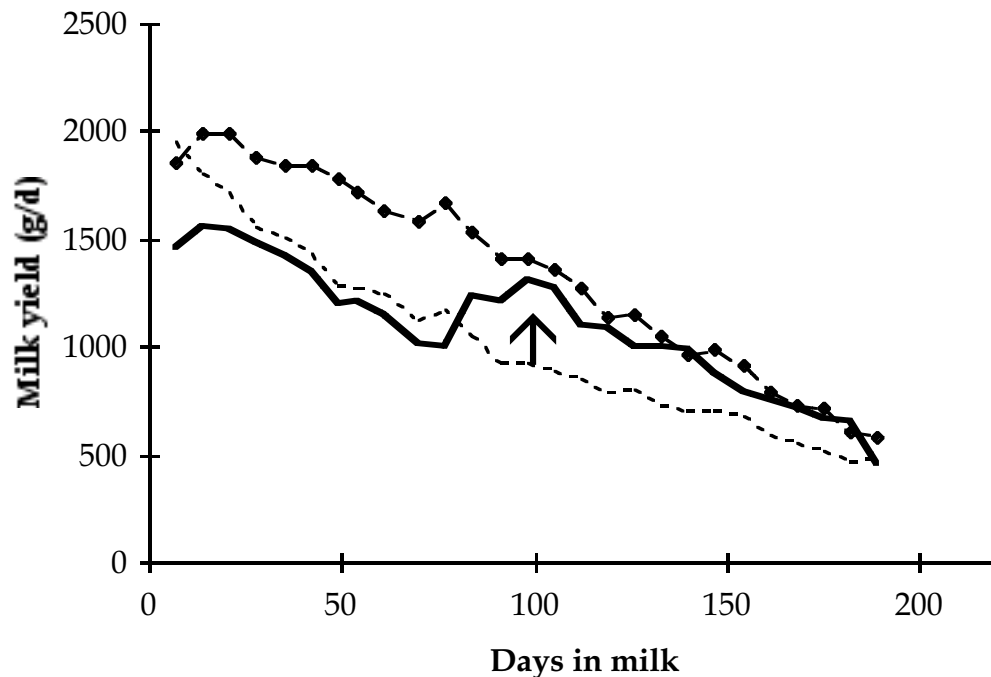


Figure 2 - Lactation curves of Mediterranean sheep. The arrow indicates a “false” lactation peak (continuous line curve) that usually occurs in the spring, when a large amount of pasture is available after a period of scarce availability (Cappio-Borlino et al., 2002). The highest curve is that of well-fed sheep and the dotted one represents a lactation curve without peak.

The lactation peak is expected within 3-4 weeks after lambing, i.e. in winter for mature ewes, but it is often smoothed due to adverse environmental conditions, such as low temperatures and scarce feed availability. On the other hand, the favorable climatic conditions that can be found during spring and, especially, the large availability of pasture usually result in a “false” lactation peak in the second half of lactation (Figure 2). This behavior has also been observed in dairy cattle under grazing management systems (Garcia and Holmes, 2001).

A common feature of dairy sheep lactation curves (30 to 50% of the cases) is the absence of the lactation peak (Cappio-Borlino et al., 1997a). Such a dimorphism could be the result of genetic and nutritional causes, because the diet is unable to meet the nutritional requirements of the ewe. Similar lactation patterns have been observed in dairy cattle (Broster and Broster, 1984; Shanks et al., 1981).

## PHYSIOLOGY OF 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 secretory epithelium is the critical point determining persistency of lactation and total milk yield. Knowledge of the physiological and environmental factors that influence the number and the activity of mammary secretory cells is necessary to determine a proper strategy for maintaining lactation.

As lactation progresses, the secretory cells gradually regress from a state of active synthesis and secretion to a non-secretory state through a process called “involution”. Considering the typical lactation curve (Figure 1), gradual involution starts around peak lactation and continues up to the time when the animals are dried off. Because milk yield is the result of the number of secretory cells and of the secretory rate of each cell, gradual involution takes place through a decrease in the number of mammary cells or a decrease in their activity. Maintenance of milk synthesis and secretion is controlled by a balance of both systemic and local regulatory factors.

### Systemic factors

The systemic factors involve hormones such as prolactin (PRL) and growth hormone (GH) that have an important role in maintaining the lactation; changes in their circulating levels affect the rate of secretion of milk. As lactation progresses, the levels of GH (Akers, 2002) and PRL (McMurtry et al., 1975) decrease, causing a reduction of milk synthesis. The influence of these hormones in reducing involution is mediated by the insulin-like growth factor-I (**IGF-I**) (Tonner et al., 2000), a hormone which is well known to increase milk yield.

The GH, whose receptors are not present in the mammary gland, exerts its positive effects on milk yield indirectly by stimulating IGF-I synthesis and secretion (Figure 3); IGF-I receptors have been identified in the mammary gland of sheep (Akers, 2002). On the other hand, the positive action of PRL in reducing involution of mammary cells is related to the suppression of insulin-like growth factor binding protein-5 (IGFBP-5) production in the mammary tissue, which

otherwise would inhibit IGF-I secretion (Tonner et al., 2000). The secretion of IGF-I is regulated by the nutritional status of animals. For example, plasma IGF-I concentration increased when the intake of high-energy and high-protein diets increased (McGuire et al., 1992). Both the increase of the frequency of feeding with concentrate supplements from one to three times a day and the improvement of forage quality increased plasma IGF-I concentration in ewes in late pregnancy (Chestnutt and Wylie, 1995).

**Local factors**

In addition to systemic factors, local factors, such as the feedback inhibitor of lactation (FIL) and the plasminogen-plasmin system, are involved in the involution process.

*Feedback inhibitor of lactation*

The accumulation of milk in the mammary gland can accelerate the involution process and reduce lactation persistency. In sheep, we observed that hourly milk secretion rates tend to decrease as the milk in the mammary gland accumulates (Figure 4; Nudda A. and Pulina G., unpublished).

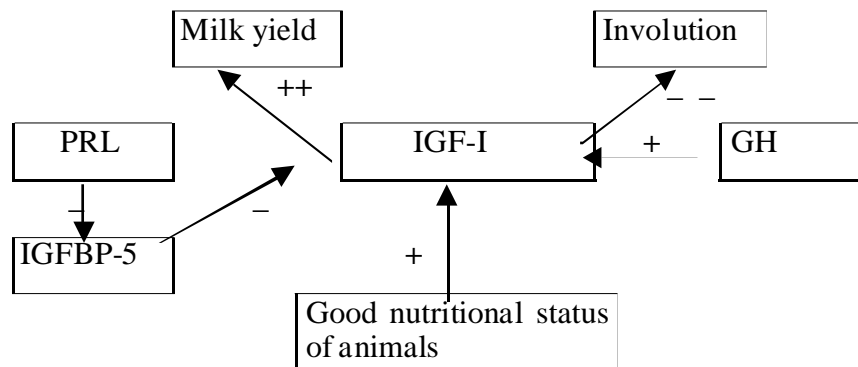


Figure 3 - A schematic description of the systemic factors that influence secretory cells activity and milk yield (+ positive effect; - negative effect).

The factor involved in the reduction of milk secretion has been identified by Wilde et al. (1987) as a peptide named feedback inhibitor of lactation (FIL), synthesized by mammary epithelial cells and secreted with the milk into the alveoli. As time from last milking increases, milk accumulates in the alveoli with this peptide, which causes the progressive reduction of milk synthesis and secretion. Therefore, frequent removal of milk (and consequently of the FIL) from the mammary gland reduces local inhibitory effects. The capacity of the mammary cistern can also have an important role in the reduction of local inhibitory effects on milk secretion due to milk accumulation with extended milking intervals. For example, ewes that stored a large proportion of milk in the cistern were able to tolerate the extension of milking interval up to 16 hours without reduction of milk secretion (McKusick et al., 2002; Pulina et al., 2002).

The local regulation of milk synthesis and secretion was firstly observed in goats (Wilde and Knight, 1990) and cows (Stelwagen and Knight, 1997), in which unilateral alteration of the frequency of milking affected only the treated gland: the increase of milking frequency from 2 to 3 times per day in one udder increased milk yield without effects on the milk yield of the controlateral gland, which continued to be milked normally (twice per day).

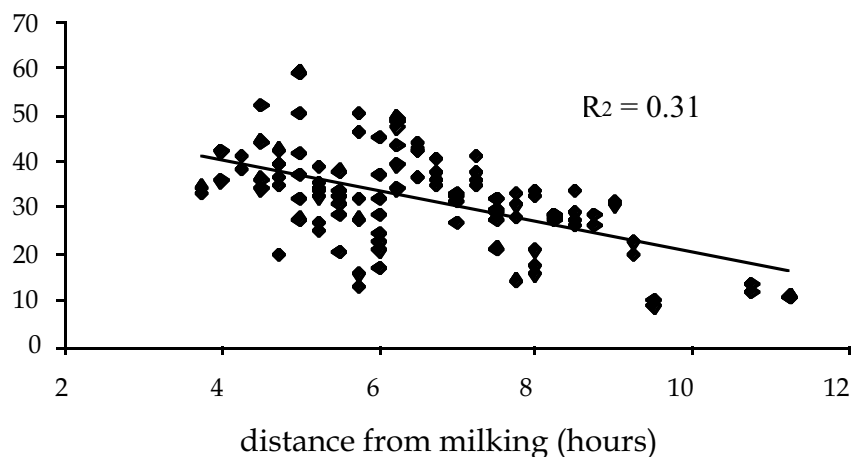


Figure 4 – Reduction of milk secretion rates due to the increase of the interval between milkings in secondiparous Sarda ewes (Nudda A. and Pulina G., unpublished).

The local regulation of milk secretion was also recently observed in dairy ewes in which the unilateral reduction of the milking frequency from twice to once a day affected only the treated gland (Nudda et al., 2002a). An important observation in this experiment was that the gland milked twice per day did not show an increase in milk production due to the greater availability of nutrient supply caused by the suppression of one milking in the opposite gland. Thus, availability of substrates for milk synthesis was not the only limiting factor in milk synthesis. This hypothesis is fully confirmed by our recent experiment on well-fed primiparous ewes in which one udder half was milked twice a day, whereas in the opposite udder half milking was completely suppressed for one week. The milk yield of this 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 - unpublished; Figure 5). In brief, the increase in availability of precursors (which were not utilized by the udder half in which milking was suppressed) did not cause an increase of their uptake for milk synthesis by the regularly milked udder half. Because the nutrients available were sufficiently high to satisfy the maximum potential synthetic rate of each cell, we concluded that milk yield was conditioned mainly by the number of cells active at the moment in which the experiment was carried out rather than the synthetic rate of each cell.

In practice, the regulation of milking frequency and of the interval between milkings in well-fed animals can be a way to manipulate milk secretion and retard the involution of secretory cells.

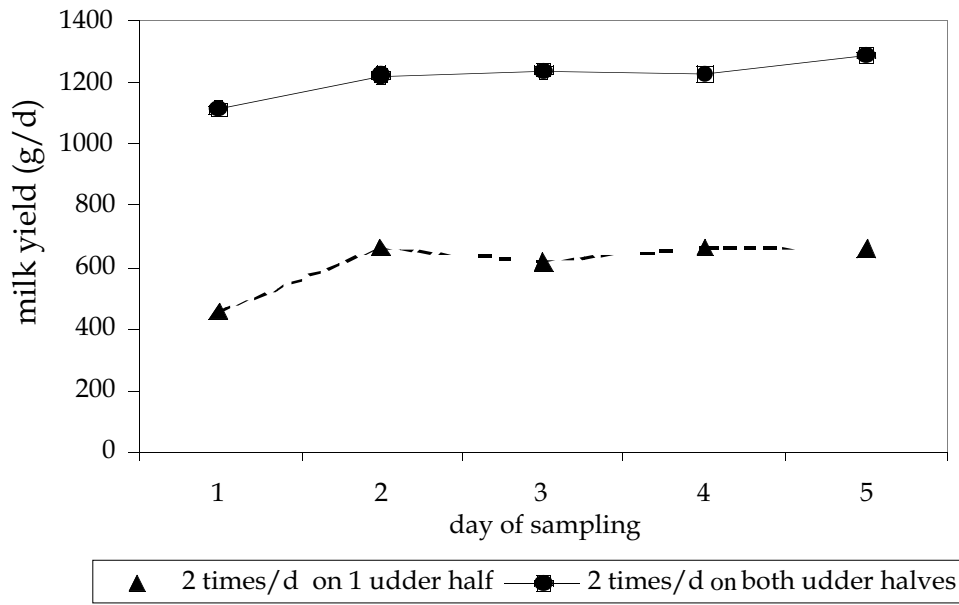


Figure 5 - Milk yield of mammary glands in which both udder halves were milked twice a day or only one udder half was milked twice a day and the other was not milked (Nudda A. and Pulina G., unpublished data).

#### *Plasmin-plasminogen system*

Plasmin is the predominant protease in milk, and it is mainly associated to casein micelles, which represent its substrate of action. Plasmin is responsible for the hydrolysis of  $\alpha$  and  $\beta$  casein in milk. Both 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. A simplified model of the processes that involve the plasmin-plasminogen system in the involution of secretory cells is shown in Figure 6.

*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 play a role in decreasing PA, probably throughout 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. The nutritional status of the animals also plays an indirect role in regulating PAI. Nutrition can influence the involution process through the regulation of plasma IGF-I concentration which, as said before, increases in well-fed animals.

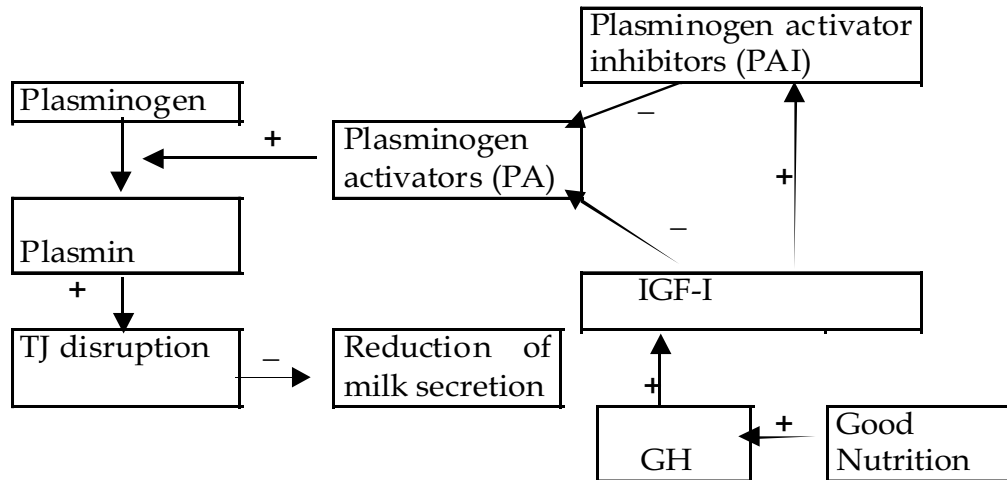


Figure 6 – A schematic representation of the mechanisms of action of the plasminogen-plasmin system on cellular involution.

*Disruption of tight junctions integrity* The involution of mammary secretory cells is triggered by the disruption of tight junctions (TJ) between adjacent cells. A schematic representation of the mechanisms involved in TJ disruption is presented in Figure 7. The TJ are structures which encircle the cells and fuse adjacent cell membranes 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 passing through the secretory cells (the transcellular route). During involution (but also in other conditions such as pregnancy, presence of mastitis, and extended milking interval) the TJ become leaky and permit the passage among cells of blood precursors that have to reach the alveolar lumen (the paracellular route). Because of the above mentioned connections between TJ and cytoskeleton, the leakiness of TJ 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 can predispose secretory cells to involution.

The impairment of TJ, which cause the activation of the paracellular pathway, allows the passage of substances among 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 when 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); the stretching caused by excessive accumulation of milk (Mephram, 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.

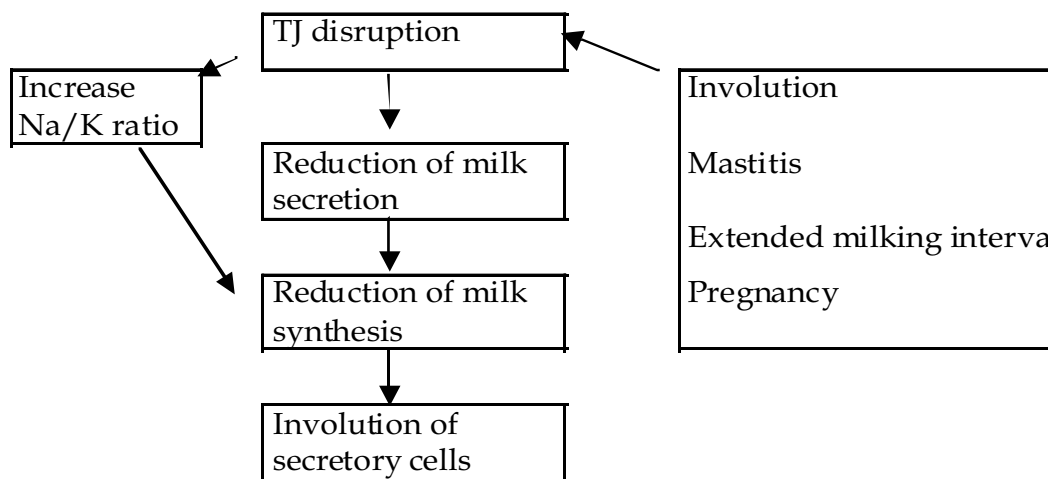


Figure 7 - A schematic and simplified representation of the mechanisms associated to tight junction disruption during cellular involution.

## NON-NUTRITIONAL FACTORS AFFECTING PERSISTENCY OF LACTATION

A proper definition of nutritional strategies to improve lactation persistency requires that the non-nutritional factors that affect lactation persistency are well defined.

### Genetics

In the present scenario of animal breeding strategies, there is a renewed interest in the persistency of lactation because the maximization of yields does not necessarily represents the best economical choice. The improvement of profitability of animal production through the reduction of production costs seems to be a reasonable option. A relevant drawback for the practical implementation of persistency as a breeding goal in dairy species breeds is represented by the great difficulty in identifying an objective measure of this trait. The several approaches that have been suggested for measuring persistency of lactation can be divided into (Gengler, 1996; Grossman et al., 1999; Jamrozik et al., 1998; Solkner and Fuchs, 1987): i) measures referring to different combinations of parameters of mathematical functions used to fit lactation curves, ii) measures based on ratios between accumulated yields and iii) measures derived from variations of test day yields; iv) days in which a constant level of production is maintained. However, none of these measurements became the standard method. Recently, a multivariate approach has been suggested to estimate an objective quantitative index of lactation persistency (Macciotta et al., 2002). Whatever index is used, the heritability of lactation persistency is low to moderate (0.10-0.30), indicating the possibility of selecting for the shape of the lactation curve (Chang et al., 2001). However, this should be made by carefully considering the relationships between persistency and yield: some results seem to indicate that the flatter the curve, the lower the total yield (Ferris et al., 1985); however, at the same level of production, relevant differences in the shape of the lactation curves were found (Macciotta et al., 2002). Thus, a reasonable approach to the selection of lactation persistency should start from the definition of an aggregate genotype that includes level of production, shape of the lactation curve and, possibly, udder morphology.

## Lambing season

The effects of the lambing season on persistency have been attributed mainly to seasonal differences in pasture availability and quality.

In Mediterranean regions, milk yield was highest in ewes that were pregnant during the spring, when there is high pasture availability (Cappio-Borlino et al., 1997b). High pasture availability had a positive effect on the differentiation of udder secretory cells and on the accumulation of body reserves during sheep pregnancy (Chestnutt and Wylie, 1995).

A superimposed effect of forage availability is the photoperiod length. In Mediterranean areas the lactation occurs during the period in which day length increases. As observed in dairy cows, the increase of 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 higher feeding activity of animals exposed to supplementary light. Indeed, sheep submitted, for a short period, to sharp changes in day length had a depression in milk production (Pulina et al., 2002).

## Parity order

The persistency of lactation was higher for ewes during first lactation compared to subsequent lactations (Figure 8). This was observed in the Valle del Belice (Cappio-Borlino et al., 1997b), Sarda (Carta et al., 1995) and Lacaune (Barillet, 1985) dairy ewes. Lesser effects occur in later lactations because persistency is in general negatively correlated with peak milk yield.

Stanton et al (1992), who observed the same effects in dairy cows, suggested that this could be explained by the maturation process, which is still in progress during the first lactation. This effect is well evident until 120 days in milk, after which maturation is gradually reduced and results in lactation curves similar to those of later lactations.

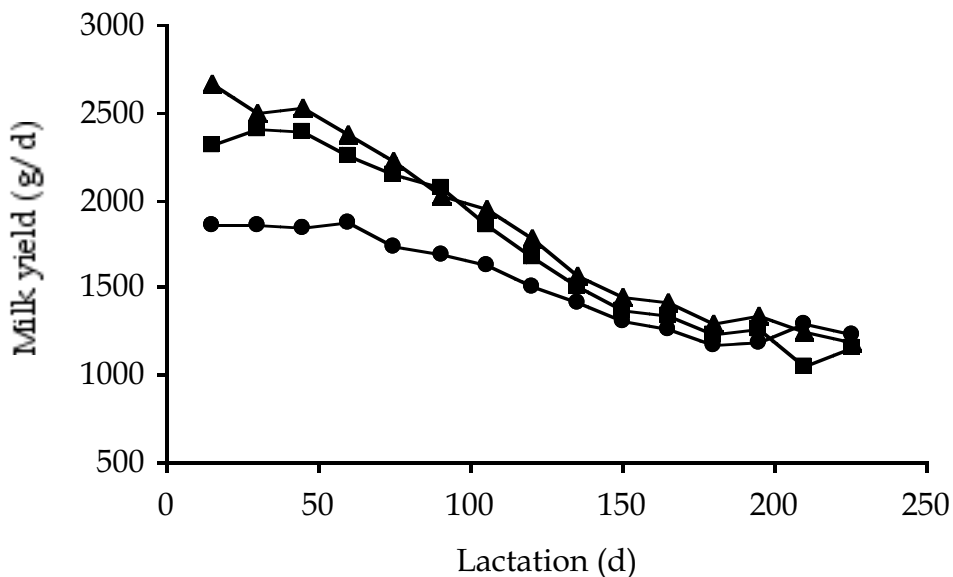


Figure 8 - Lactation curves in ewes of different parity (1 (●), 2 (■), and ≥3 (▲)); (Cappio-Borlino et al., 1997b).

### Type of lambing

Several studies have reported higher milk yield for ewes with multiple births (Table 1). This can be explained by the fact that ewes rearing multiple fetuses or with higher weight of single fetuses have higher placental weight and higher serum progesterone and placental lactogen hormones during late pregnancy (Butler et al., 1981; Schoknecht et al., 1991). These hormones stimulate greater development of the lobular alveolar structure during pregnancy and consequently greater milk production during lactation. Another factor is the more frequent suckling applied to mammary glands by twins than by one lamb, causing the removal of local inhibitors of milk secretion, such as the FIL (see previous discussion).

Table 1 – Effect of prolificacy on milk yield in different sheep breeds.

BREED	MAIN USE	MILK YIELD twins vs. single	AUTHORS
Delle Langhe	Milk	+ 10%	Ubertalle et al., 1990
Sarda	Milk	+ 11%	Pulina et al., 1993a
Churra	Milk	+ 4.5%	Gonzalo et al., 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

The data reported in Table 1 highlight that the effects of prolificacy on milk yield are higher in meat-wool sheep than in dairy ewes. In dairy ewes the higher production of ewes rearing twins is usually evident only in the first part of lactation (Figure 9) (Pulina et al., 1993a). In meat-wool sheep an effect of prolificacy on persistency has been detected along the whole curve of lactation (Figure 10) (Snowder and Glimp, 1991). However, at least part of this difference is due to a different lactation length. Indeed, in both cases the difference between twins and single lambs was evident for about 14 weeks of lactation.

The difference in milk production associated with the type of lambing can be related to different weaning techniques adopted in dairy and non-dairy sheep. In dairy sheep the lambs are usually removed from their dams after 4 weeks of lactation (Brandano and Lanza, 2002; McKusick et al., 1999a), while in meat-wool sheep the weaning starts after two or three months of lactation (Bencini, 1993; Snowder and Glimp, 1991). When weaning takes place, milk production decreases due to less frequent udder evacuation (McKusick et al., 2001). For example, the rapid decrease of the lactation curve observed in meat-wool sheep (Figure 10) between 56 and 70 days of lactation was explained by the authors by the decreasing dependence of lambs on mother's milk due to the increased grazing activity and forage intake of lambs (Snowder and Glimp, 1991).

Regarding the influence of weaning system on milk yield, McKusick et al. (1999) reported higher commercial milk production over the entire lactation in ewes whose lambs were weaned 24 hr post-partum (twice daily milking) or had access to the mother for 15 h/d (once daily milking) than ewes that had unlimited access to their lambs (no milking) for 32 days post-partum. No differences in lactation length between the three weaning systems were observed.

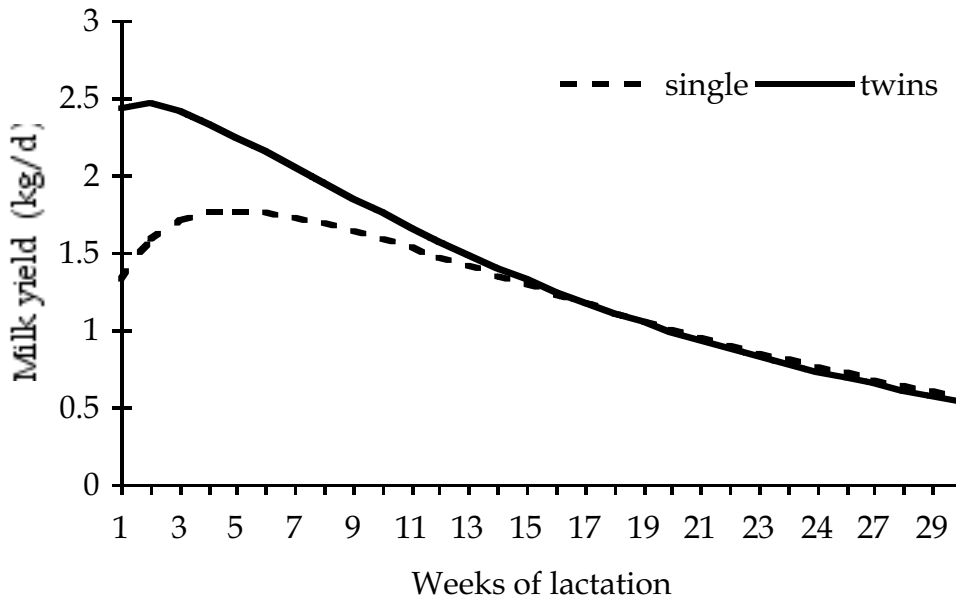


Figure 9 - Lactation curves in dairy ewes with different type of lambing (Pulina et al., 1993a).

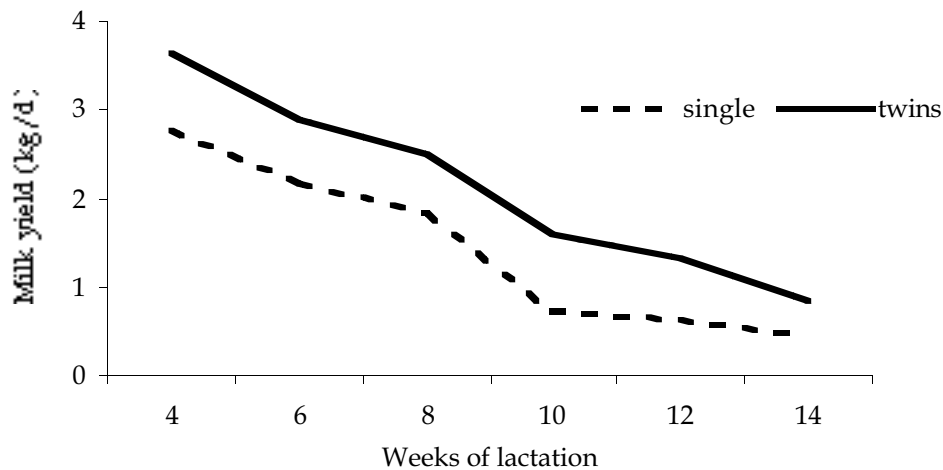


Figure 10 - Lactation curve in meat-wool sheep with different type of lambing (Snowder and Glimp, 1991).

### Milking frequency

The reduction of the milking frequency or the extension of milking intervals can accelerate the involution process and reduce lactation persistency through a mechanism that involves a local inhibitor peptide (FIL) or other systemic and local factors, as described in a previous section.

In dairy sheep, once per day milking reduced milk yield (Cannas et al., 1991; Labussière et al., 1974; Morag, 1968) in comparison with twice daily milking (Figure 11; Cannas et al., 1991) with similar intensity in dairy and meat-wool sheep breeds (Pulina and Nudda, 1996).

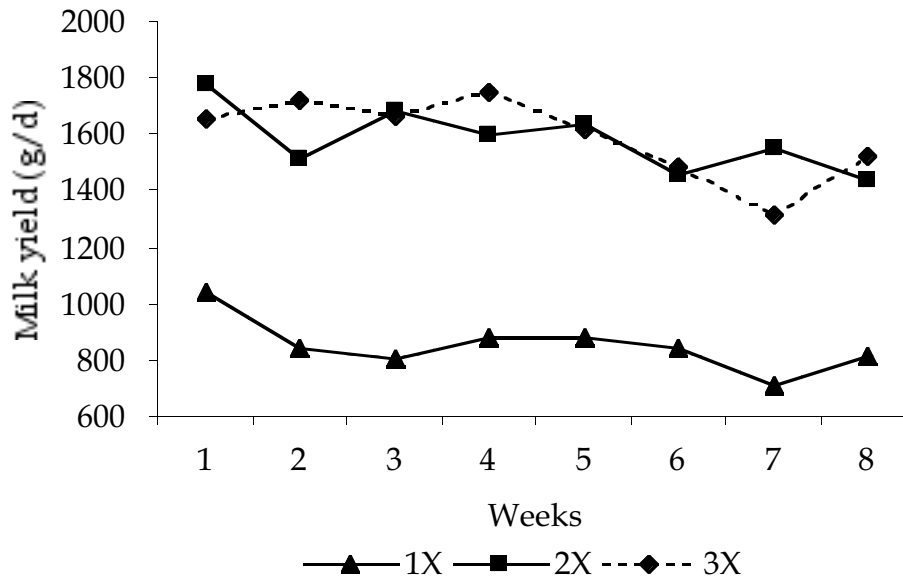


Figure 11 - Influence of milking frequency on milk yield in Sarda dairy ewes (Cannas et al., 1991)

By contrast, the effect of increased milking frequency above twice per day is higher in non-dairy than in dairy ewes (Karam et al., 1971; Bencini, 1993). This can be explained by differences in cistern storage capacity among breeds. For example, in Sarda ewes, that store a large proportion of milk in the cistern (Marnet and McKusick, 2001; Nudda et al., 2000), the increase of milking frequency from 2 times to 3 times per day had a small effect on milk yield during whole lactations (+3%; Cannas et al., 1991), while in Merino ewes the increase in milk yield was about 21% (Bencini, 1993). The difference is probably due to the smaller udder storage capacity of Merino ewes compared to Sarda ewes. Low capacity to accumulate milk during milking intervals requires more frequent milk removal from the udder, as discussed below.

### Udder morphology and cistern dimension

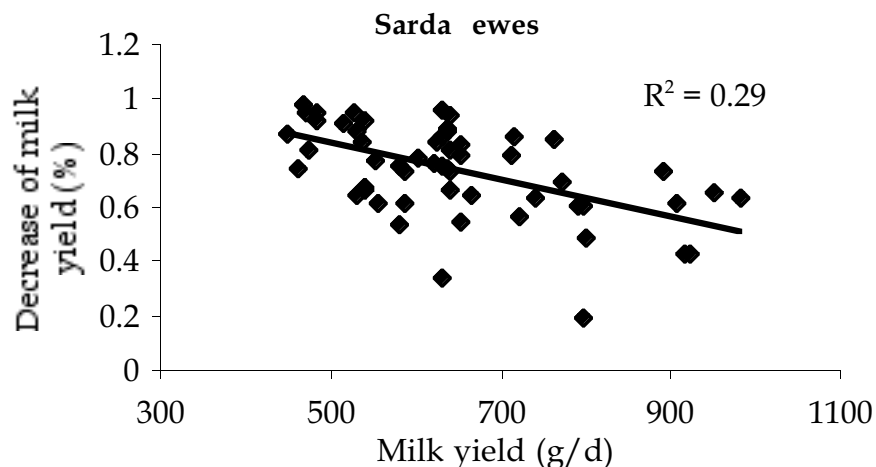
As the alveoli are the site of action of the inhibitor peptides (Henderson and Peaker, 1984), the local inhibitory factors (i.e. the FIL) affect the rate of secretion when the milk is stored in the secretory tissue, whereas they are inactive in the milk stored in the cistern. As a consequence, the action of the FIL should be of a lower magnitude in animals with a greater cistern volume, 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 (Casu and Labussière, 1972). In other studies, the relationship found between cistern size and milk yield was low and sometimes negative (Fernandez et al., 1995a; Gallego et al., 1983; Rebello de Andrade et al., 1989; Labussière et al., 1981). This lack of relationship between cistern size and milk yield can be the result of the method usually used to measure the size of the cistern. This method (Labussière et al., 1981) estimates cistern size by measuring the cistern height between the lowest udder point and the teat implantation line. However, the cistern is an internal structure of the udder that is neither visible nor measurable externally, and can only be measured using ultrasound techniques (Nudda et al., 2000b). Recent studies in which ultrasound technique was

used to measure cistern size confirm the high 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). We think that the comparisons made among breeds using the method of Labussière et al. (1981) are not reliable and do not depict the actual differences in cistern size among breeds. However, in general, non-dairy ewes have smaller cistern size than dairy ewes. This can explain their low persistency after the lambs are weaned.

The hypothesis that the action of the FIL should be of a lower magnitude in animals with a greater cistern volume has been tested in our recent experiment in which dairy and non-dairy breeds were compared. We observed that two breeds highly selected for milk production (Sarda and Awassi) responded to the reduction in the frequency of milking from twice to once a day with milk yield losses (18% to 24%) similar to those observed in Merino ewes, a wool breed not selected for milk production (Nudda et al., 2002a). A possible explanation of this result is related to the very different milk yield among the two breeds in this experiment. The ratio between milk volume and milk cistern storage capacity was probably not very different between the two breeds, explaining why the decrease in milking frequency affected them in a similar way. Indeed, the extent of milk yield reduction with once per day milking increased in proportion to the production level in the Sarda ewes (Figure 12), whereas in Merino ewes the reduction was independent from the production level (Figure 12), probably for their very low production. This is in accordance with Partearroyo and Flamant (1978), who observed, in Sarda ewes, that when passing from twice per day to once per day milking, there was a milk yield reduction of 19% in animals with milk yield higher than 600 g/d and of 2.3% only in animals with milk yield lower than 600g/d.

## NUTRITIONAL FACTORS AFFECTING LACTATION PERSISTENCY

Milk production in the first months of lactation influences the production in later months. An example of the effects of nutrition in the first part of the lactation on milk yield in the second half of lactation is reported in Figure 13 (Bomboi G., Cannas A., Molle G., unpublished).



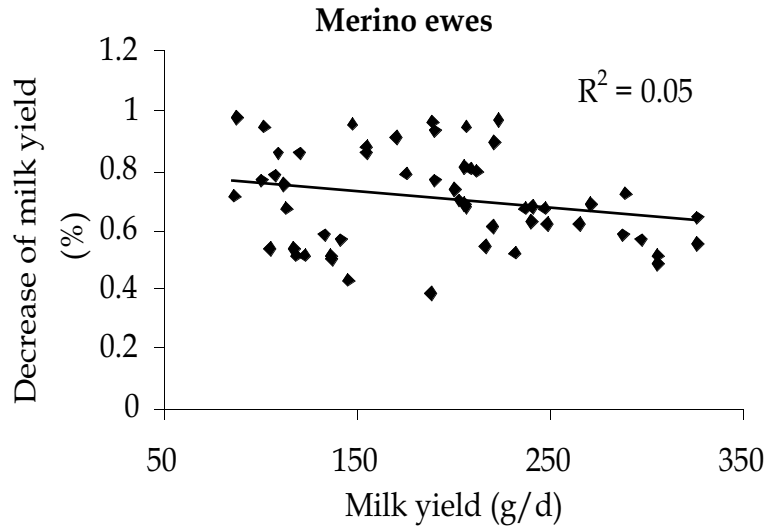


Figure 12 - Decrease in milk yield passing from two to one milkings per day according to the production level of the animals in Sarda and Merino breed (Pulina and Bencini, unpublished data).

In this experiment 60 dairy ewes were divided in 3 groups with the same mean milk yield at the beginning of the trial (9<sup>th</sup> week of lactation). Until the 20<sup>th</sup> week of lactation, each group had a different diet. One grazed ryegrass and 350 g/d of high starch concentrate as a supplement. The second group grazed the same sward but had as a supplement 350 g/d of low starch concentrate. The third one grazed on alfalfa and had as a supplement 350 g/d of low starch concentrate. The result was a significantly higher milk yield in the group that grazed alfalfa compared to the other two groups. At the 20<sup>th</sup> week of lactation all the animals were put together and fed the same diet (ryegrass pasture plus concentrate supplements). During the 10 weeks after the experiment, the group previously fed alfalfa maintained the highest milk yield and produced 210 g/d more milk than the ewes of the groups previously fed on ryegrass.

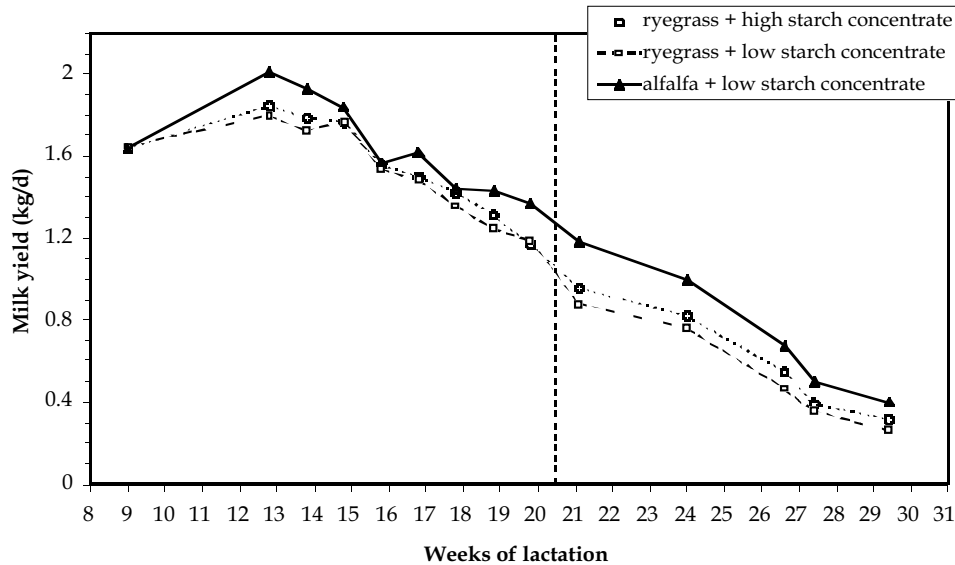


Figure 13 - Milk yield of three groups of Sarda ewes fed diets differing in the type of pasture and the quality of the supplements. The treatments were applied from the 9<sup>th</sup> to the 20<sup>th</sup> week of lactation. From the 21<sup>st</sup> to the 30<sup>th</sup> week of lactation all the ewes grazed the same pasture and received the same supplements (Bomboi G., Cannas A., Molle G., unpublished). The dotted line separates the two periods.

Due to the importance of milk production in the first part of the lactation on lactation persistency, the main nutritional strategies to improve milk yield in this phase will be discussed.

### **Nutrition during pregnancy and effect of body reserves on milk yield**

Proper nutrition during pregnancy favors the development of the secretory tissue of the mammary gland, probably as a result of the action of the placental lactogen hormone secreted by the placenta during the pregnancy. The effect is an increase in the number of mammary secretory cells and thus a higher potential milk yield. An example of the effect of nutrition during pregnancy is given by the experiment of Bizelis et al. (2000) and Charismiadou et al. (2000), who fed pregnant sheep with two different levels of nutrition (high: 110% of energy requirements; low: 90% of energy requirements). They found that the ewes fed the high level of nutrition had a larger udder at the 140<sup>th</sup> day of pregnancy (Table 2) and a higher number of secretory cells (higher DNA content).

Table 2 - Effects of two feeding levels (FL) (high: 110% of requirements; low: 90% of requirements) during pregnancy on udder characteristics at 140<sup>th</sup> d of pregnancy and on milk yield during lactation (Charismiadou et al., 2000; Bizelis et al., 2000).

	<b>High FL</b>	<b>Low FL</b>
Udder weight (kg)	2.08	1.46
Udder circumference (cm)	44.4	38.4
Teat length (cm)	4.9	4.3
Mammary gland weight (kg)	1.55	1.15
Total DNA (g)	4.97	2.82
Total RNA (g)	4.97	2.82
Total milk yield (l to 12 weeks of lactation)	114	82
Daily milk yield (l/d)	1.357	0.976
BW variations (kg in first 7 weeks of lactation)	0	+2.4

In the same experiment, after lambing all the ewes were put together and fed the same diet *ad libitum* for 12 weeks of lactation. The intake of energy during lactation was slightly higher in the first days of lactation for the ewes fed the low level of nutrition during pregnancy. After this, there were no differences in intake. Milk yield was significantly higher in the ewes that had the highest level of nutrition during pregnancy (Table 2). This was the result of the larger number of mammary secretory cells but probably was due to the larger availability of body reserves during lactation also.

Mammary gland development can also be stimulated by hormonal treatments. For example, sheep treated with prostaglandin and then with pregnant mare serum gonadotropin at the end of the diestrus had larger mammary gland and higher number of secretory cells than untreated animals. This resulted in a dramatically higher (+59%) milk yield in the first 4 months of lactation compared to control ewes (Manalu et al., 2000).

Unfortunately neither of the two studies cited above measured milk yield after the first 4 months of lactation. Thus, possible effects on persistency could not be evaluated.



Proper nutrition during pregnancy also influences milk yield because it allows the accumulation of sufficient body fat and protein reserves, which can be mobilized in the first months of lactation. Even when sheep are fed high quality diets, in the first months of lactation a negative energy balance is inevitable. This occurs because after lambing, milk production and, consequently, the energy requirements of the animals grow more rapidly than the intake of energy from the diet. This situation is worsened when the first months of lactation coincide with low feed availability (e.g. winter lambing season). In the first two months sheep produce a large part of their milk by mobilizing their body fat and protein reserves. Caja and Bocquier (1998) reported that at the beginning of the lactation almost 50% of milk energy came from the mobilization of sheep body reserves.

Thus, it is very important that sheep have sufficient body reserves at the beginning of lactation. Robinson (1987b) has clearly shown how body fat reserves and energy intake are of great importance in this period. In his experiments, milk production of sheep with very high energy intake was almost unrelated to body reserves. However, when the energy intake was lower, milk production was greatly influenced by sheep body fat reserves. The thinner the sheep, the less milk that was produced. Given that the highest energy intake reported by Robinson (1987b) is hard to achieve in grazing sheep, because concentrates are supplied separately from forage, and low forage to concentrate ratios are not feasible, it becomes obvious that sufficient fat reserves are essential for high milk production in the first months of lactation.

Body reserve losses can be considered normal as long as they are not excessive or too fast. In BCS terms, the INRA (1989) suggests an optimal body condition score (**BCS**) at lambing between 3.25 to 3.5, which may fall to a minimum of 2.0 to 2.5 in the 6<sup>th</sup> to 8<sup>th</sup> week of lactation. The ewes should not lose more than 1 BCS in six weeks. Too high energy deficits cause reduction of milk production (Caja and Bocquier, 1998), with increased risks of ketosis and a negative effect on milk yield in the second part of the lactation.

Lactating ewes with highly negative energy balance tend to reduce their milk production more markedly than cows do. Indeed, while in cows genetic selection has resulted in dairy cows having a hormonal status that encourages milk production even with high fat mobilization, in dairy sheep there has not been so much genetic improvement, and ancestral characteristics designed to protect the life of the animal are often more evident. Even in cattle, animals of breeds not selected for milk production decrease their milk yield more rapidly when they are underfed compared to dairy cattle (Preston and Leng, 1986; cited by Chilliard, 1992).

However, ewes that are too fat at lambing usually have low milk production in the first months of lactation. Stern et al. (1978) observed that ewes overfed during pregnancy had markedly lower DM intake (-21%) and milk yield than ewes properly fed during pregnancy. This happened because the excessive quantity of visceral fat compressed the rumen and reduced feed intake and nutrient availability.

### **Monitoring sheep energy balance**

During the first months of lactation it is important to monitor the energy balance of the ewes and to avoid it becoming too negative. This is particularly important in sheep because the high

genetic variability within breed causes large variability in milk yield as well. An example is given in Figure 14, where the distribution of milk yields in a flock of Sarda ewes in the 4<sup>th</sup> month of lactation is represented. This high variability implies that for a proper feeding management the flock of a farm should be subdivided in subgroups based on the milk yield of the ewes. This should be done to avoid underfeeding of high productive animals and overfeeding of the least productive animals. Group feeding strategies have been studied by Caja and Bocquier (1988) and by Bocquier et al. (1995).

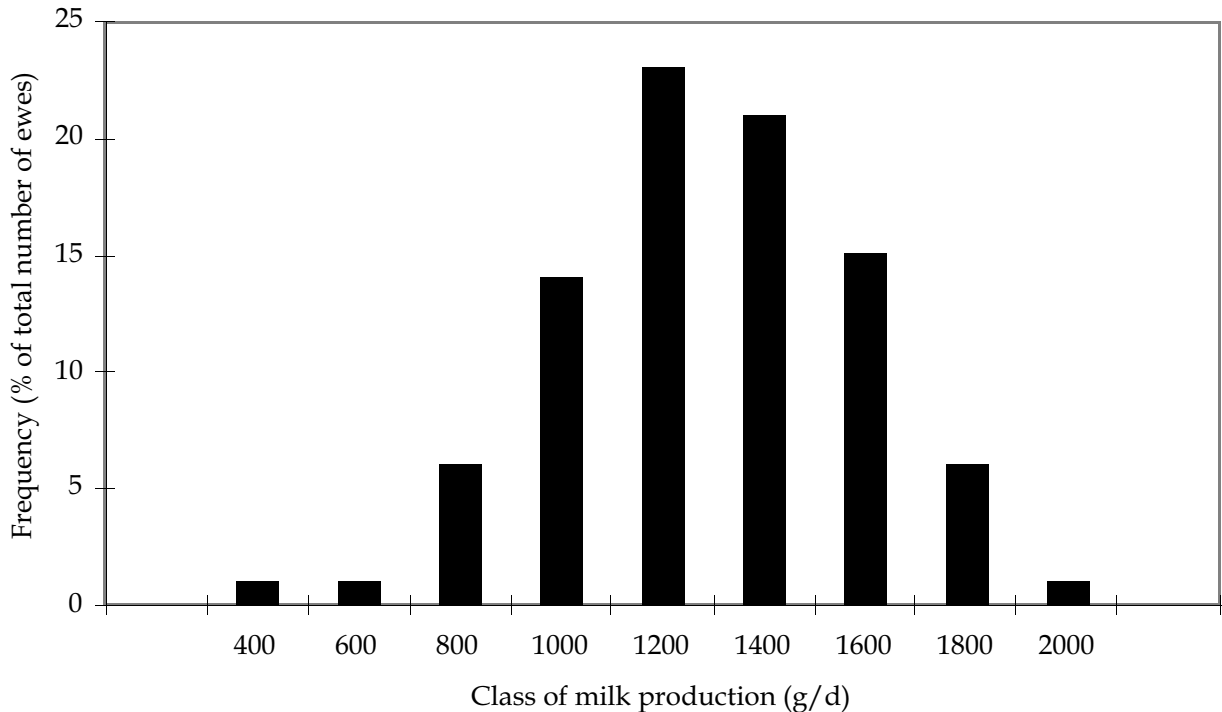


Figure 14 - Distribution of milk yields within a flock of lactating Sarda ewes in the 4<sup>th</sup> month of lactation.

One tool that can be used in the field to monitor the energy balance of the ewes is to assess their BCS. This method is fairly accurate when the mean BCS variation of a flock needs to be monitored. However, it cannot be easily applied to identify those animals within the flock that are losing weight too quickly.

Another possible method involves the measurement of the variations of milk fat concentration during lactation. Sheep energy balance (**EB**) is probably the factor that has the largest effect on milk fat concentration. Indeed, when body fat mobilization is high (usually at beginning of the lactation), the effect is an increase of the blood concentration of long chain fatty acids derived from body fat triglycerides; part of these fatty acids are used by the mammary gland to produce milk fat, whose concentration increases. Another effect is an increase in the concentration of long chain fatty acids in milk fat. Bocquier and Caja (1993; 2001) found a close negative relationship between EB and milk fat concentration, in sheep of different dairy and non-dairy breeds with milk yields ranging between 0.65 and 3.5 l/d:

$$\text{milk fat concentration (weight/volume)} = 6.84 - 1.22 \text{ EB (UFL/d)}; r^2 = 0.76$$

where UFL = 1.7 Mcal of NEL<sub>1M</sub>.

Recently, Cannas and Avondo (2002) observed a similar relationship in grazing Comisana dairy ewes with milk yields of between 0.65-1.60 kg/d. This suggests that the relationship between milk fat concentration and EB is not markedly affected by the breed of the ewes. Cannas and Avondo (2002) subdivided their database into 4 classes, which were based on the milk yield of the ewes (< 400 g/d, n = 63; 400-799 g/d, n = 260; 800-1199 g/d, n = 97; 1200-1600 g/d, n = 20) and noted that, going from the highest to the lowest milk yield class, this relationship became weaker (Figure 15). It is clear that the relationship between milk fat concentration and EB is especially important for ewes with high milk yield, while it is very weak for animals with low milk yield.

In any case, as EB varied, the range of variation in milk fat concentration found by Bocquier and Caja (1993; 2001) and by Cannas and Avondo (2002) in sheep was much larger than that observed in dairy cows (e.g. Grieve et al., 1986), suggesting a more important contribution of FA derived from body fat mobilization in sheep than in cows.

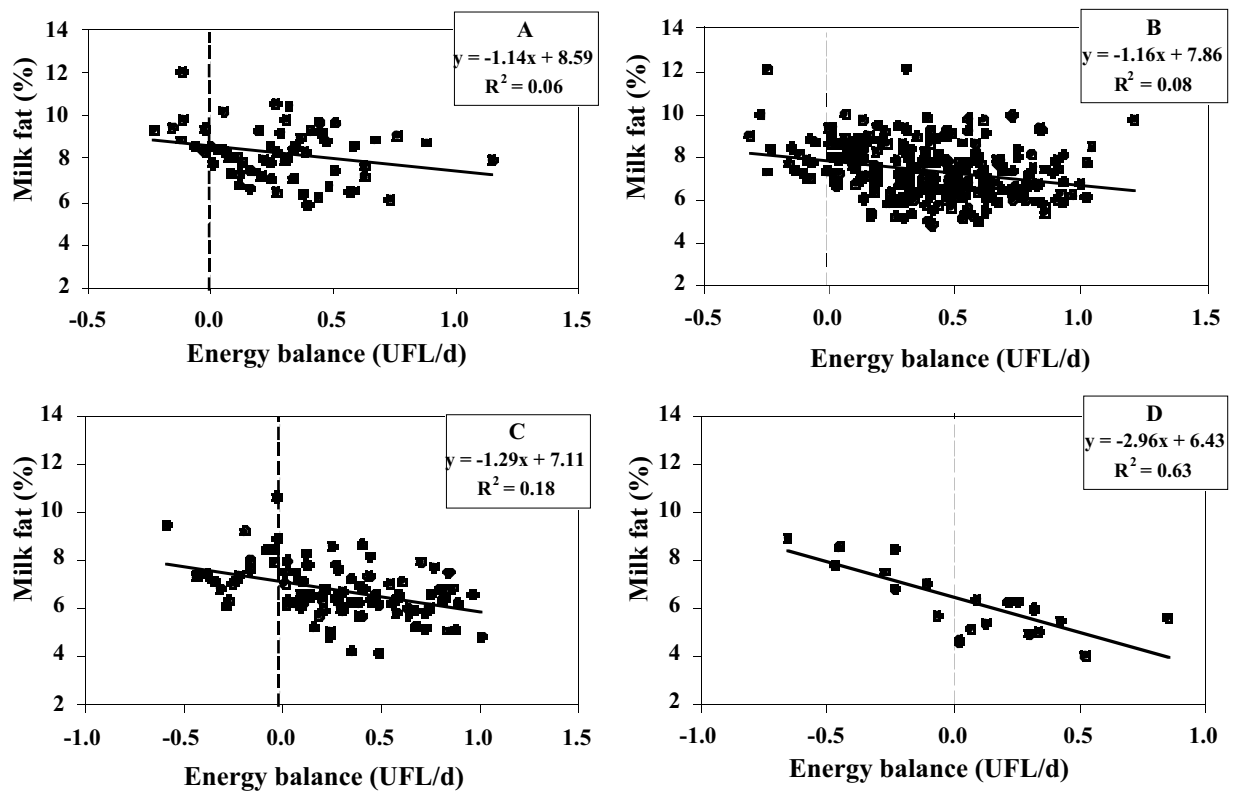


Figure 15 - Relationship between milk fat concentration and energy balance of grazing Comisana ewes divided into 4 milk yield production classes: A = < 400 g/d, n = 63; 400-799 g/d, n = 260; 800-1199 g/d, n = 97; 1200-1600 g/d, n = 20. The dotted line represents, in each plot, the limit between negative and positive energy balance (adapted, from Cannas and Avondo 2002). All the regressions were significant for  $P < 0.01$ , except for the regression A for which  $P < 0.1$ .

The close relationship between EB and milk fat content suggests that milk fat concentration can be used to predict the EB of the animals. Similarly, in dairy cows the variations in milk fat concentration during lactation were used to identify across-herd differences in EB and to identify potential problematic cows within the herd (de Vries and Veerkamp, 2000), such as animals that are losing body fat reserves too quickly and are probably in sub-ketosis.

It seems, then, that milk fat concentration and its variations can be used as an nutritional indicator in the field, even though more research is needed to increase its accuracy.

### **Nutrition during the first part of the lactation**

In sheep, the effect of dietary energy concentration seems to be markedly affected by the stage of lactation.

During the first part of lactation, energy rich diets generally gave much higher milk yield than medium-low energy concentration diets (Susin et al., 1995; Abdel-Rahman and Mehaia, 1996; Al Jassim et al., 1999; Caja and Bocquier, 2000; Alexandre et al., 2001). For example, high roughage diets (60:40 forage to concentrate) gave much lower milk yield than low roughage diets (20:80 forage to concentrate) in Finnsheep ewes in the first weeks of lactation (Brown and Hogue, 1985) (Table 3).

While the ewes fed rich energy diets increased their milk yield in the first 7 weeks of lactation, the opposite occurred in the ewes fed high fiber diets.

Table 3 - Effect of the forage to concentrate ratio on milk yield (kg/d) in the first weeks of lactation of Finn sheep ewes (Brown and Hogue, 1985).

	<b>Forage to concentrate ratio</b>	
	<b>60:40</b>	<b>20:80</b>
<i>Week 2</i>	1.45	1.54
<i>Week 4</i>	1.15 <sup>a</sup>	1.78 <sup>b</sup>
<i>Week 7</i>	1.04 <sup>a</sup>	2.71 <sup>b</sup>
<i>Mean</i>	1.21 <sup>A</sup>	2.11 <sup>B</sup>

<sup>a, b</sup> P < 0.05    <sup>A, B</sup> P < 0.01

Under-nutrition in the first part of lactation generally results in low and delayed peaks of milk yield or the absence of the lactation peak (continuously declining curves) (Figure 2). Under-nutrition in this stage, especially when it is prolonged, reduces the number of secretory cells permanently. Despite this, re-feeding can induce higher milk yield than observed during the under-nutrition period (Caja and Bocquier, 2000). This probably happens because the secretory cells that are still active increase their synthetic activity.

### **Nutrition during the intermediate and final part of the lactation**

In nutritional terms an exact definition of the moment of transition from initial lactation to full lactation should be based on whether or not the animals have a negative (first phase) or positive (second phase) energy balance rather than on the month of

lactation. When the ewes are well fed, this transition can occur very early (during the first two months of lactation). However, when the nutrition of the ewes is not adequate the transition is postponed. For example, in the Mediterranean region it occurs after the beginning of the Spring, when the ewes are in their 3<sup>rd</sup>-4<sup>th</sup> month of lactation and have their peak of lactation.

In contrast with the first part of the lactation, the utilization of diets rich in sugars and starch in this stage gives controversial results. There are cases in which the utilization of concentrates rich in starch and protein markedly improves milk yield even in late lactation. This usually happens when the diet is markedly unbalanced or when feed intake is low, such as when the ewes are fed on mature or scarce pastures. As an example, we report an experiment carried out at the end of the lactation in Sarda sheep grazing on cereal stubbles, which in the Mediterranean areas are usually very rich in fiber (60-70% of NDF, DM basis) and low in protein (6%-8%, DM basis). Supplementing the diet with concentrates rich in starch and protein increased milk yield and lactation persistency (Figure 16).

When the diets are sufficiently well balanced, the effects of nutritional treatments in mid-late lactation are often more evident on BW variations (usually positive) than on milk yield (often unchanged or reduced) (Bocquier and Caja, 1993).

An example is given by the results of an experiment carried out during the 5<sup>th</sup> and the 6<sup>th</sup> month of lactation of Sarda ewes (Bomboi et al., 2002) (Tables 4 and 5). During the preliminary period the ewes were fed a TMR diet with a 75:25 forage to concentrate ratio, made of chopped alfalfa and concentrates (Table 4).

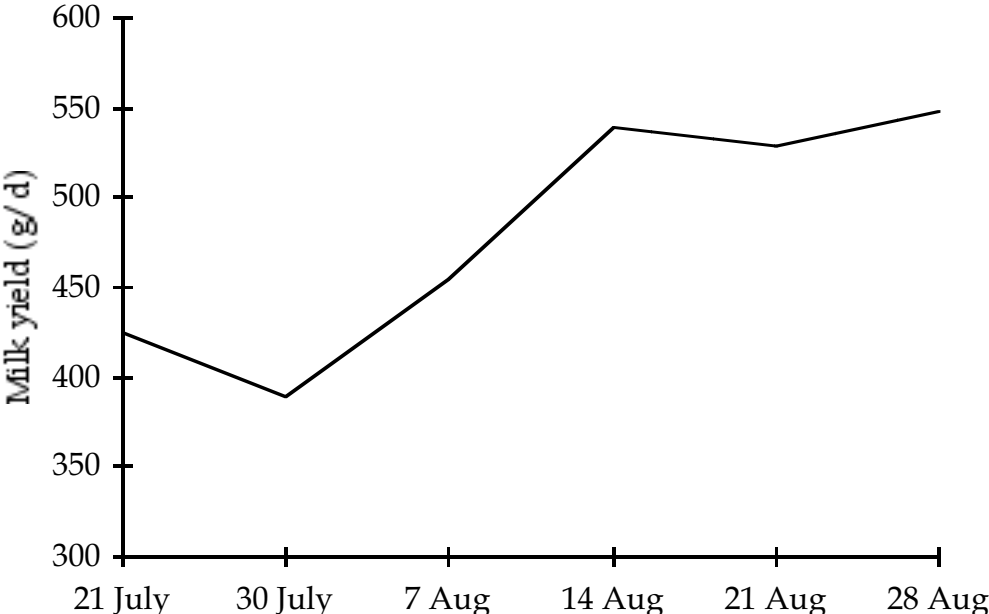


Figure 16 – Positive effects of the supplementation with concentrates rich in energy and proteins on milk yield of dairy ewes in late lactation (8<sup>th</sup> - 9<sup>th</sup> month) fed on stubble (Pulina et al., 1993b).

During the 7 weeks of the experimental period, the ewes were subdivided in three groups. One group kept the preliminary period diet, another was fed a diet rich in forage (90:10 forage to concentrate ratio) and the third one was fed a diet rich in concentrates (60:40 forage to concentrate ratio). The results (Table 5) showed that DM intake was the lowest with the 90:10 diet, and energy intake increased as the forage to concentrate ratio decreased. Milk yield was not affected by the feeding treatments, while BW significantly increased as the content of concentrate in the diet increased. This experiment suggests that the extra energy received by the ewes when the percentage of concentrates in the diet increased (from 25% to 40% of the diet) was only used to accumulate body reserves. By contrast, when the forage content increased (from 75% to 90% of the diet), despite the lower intake of energy, milk yield was similar to that of the 75:25 group, suggesting that a larger proportion of dietary energy was used for milk production.

Comparing Greek dairy ewes fed diets with two very low forage to concentrate ratios (40:60 vs. 20:80), which also differed in the fiber content of the concentrates, during the 4<sup>th</sup> and the 5<sup>th</sup> month of lactation, Zervas et al. (1999) observed a significant but small increase in milk yield for the diets with the lowest ratio. However, these diets were quite low in CP concentration. In addition, there were large differences in dietary CP content among some of the treatments. This may have affected the results. The same comment can be made about the experiment of Goodchild et al. (1999), in which Awassi ewes were fed diets differing in forage to concentrate ratio but with large differences in dietary CP concentration. Since dietary protein can markedly affect milk yield (e.g. Figure 17) and DM intake (Van Soest, 1994), comparisons should always be made with isonitrogenous diets.

Table 4 - Chemical composition and ingredients of an experiment in which lactating Sarda ewes in mid-lactation were fed diets differing in the forage to concentrate ratio (Bomboi et al., 2002).

	<b>FORAGE TO CONCENTRATE RATIO</b>		
	<b>90:10</b>	<b>75:25</b>	<b>60:40</b>
<b><i>Ingredients (g/kg of DM)</i></b>			
Chopped alfalfa hay	900	750	600
Rolled barley grain	37.5	94	150
Rolled corn grain	37.5	94	150
Soybean meal	22	55	88
Minerals + vitamins	3	7	12
<b><i>Composition</i></b>			
CP (% of DM)	17.3	17.5	17.6
NDF (% of DM)	35.2	31.1	27.1
NFC <sup>1</sup> (% of DM)	37.4	41.8	46.3
NEL (Mcal/kg of DM)	1.425	1.512	1.599

<sup>1</sup> NFC = non structural carbohydrates, calculated as: 100 - (NDF-NDF<sub>IP</sub>) - CP - EE - ash.

Table 5 – Main results of an experiment in which lactating Sarda ewes in mid lactation were fed diets differing in the forage to concentrate ratio (Bomboi et al., 2002). Covariate adjusted means.

	Forage to concentrate ratio		
	90:10	75:25	60:40
DM intake (g/d)	2147 <sup>A</sup>	2262 <sup>B</sup>	2213 <sup>A<sup>B</sup></sup>
NEL intake (Mcal/d)	3.064 <sup>D</sup>	3.417 <sup>E</sup>	3.538 <sup>E</sup>
Milk yield (g/d)	1114	1110	1134
Fat (%)	5.53 <sup>AB</sup>	5.30 <sup>A</sup>	5.70 <sup>B</sup>
Protein (%)	5.62 <sup>A</sup>	5.47 <sup>B</sup>	5.82 <sup>C</sup>
Body weight (kg)	45.98 <sup>D</sup>	46.85 <sup>E</sup>	47.91 <sup>F</sup>
Glucose (mg/dl)	53.3 <sup>D</sup>	57.4 <sup>E</sup>	60.0 <sup>E</sup>
Insulin (μU/ml)	26.6	26.7	27.2
NEFA (μEq/l)	165 <sup>A</sup>	151 <sup>A</sup>	107 <sup>B</sup>
Somatotropin (ng/ml)	2.54 <sup>a</sup>	1.93 <sup>b</sup>	1.52 <sup>b</sup>

<sup>a, b, c</sup> = P < 0.07; <sup>A, B, C</sup> = P < 0.05; <sup>D, E, F</sup> = P < 0.001

In some experiments higher milk yield was observed using diets with low nonstructural carbohydrates and high fiber concentration compared to richer diets.

East Friesian ewes from the 5<sup>th</sup> to the 7<sup>th</sup> month of lactation were fed TMR diets differing in the concentration of starch and sugars (NSC) in the concentrates (Cavani et al., 1990). In one case, the concentrate consisted of a pelleted feed containing 35% of NSC, while in the other case a mixture of pelleted feed, soybean hulls and distillers of cereals containing 20% of NSC was used (Table 6). On the whole, one diet had 20.7% (DM basis) NSC, the other one 15.7% (DM basis) (Table 6). The results of the experiment showed that the two groups of ewes had a similar daily intake of energy. However, the group fed the diet with the lower NSC concentration had a significantly higher milk yield, milk fat concentration and milk fat corrected milk yield when compared to ewes fed diets with the higher NSC concentration, which in turn had higher positive BW variations (Table 7).

In another experiment, lactating Sarda ewes in mid-lactation (from the 4<sup>th</sup> to the 6<sup>th</sup> month), were fed pelleted diets with increasing concentrations of CP (from 14% to 21% CP; Table 8) at two levels of energy (HE, high energy = 1.65 Mcal/NE<sub>L</sub> kg DM; LE, low energy = 1.55 Mcal/NE<sub>L</sub> kg DM) and non fiber carbohydrates (NFC: sugars, starch, and pectins) concentration (Table 8) (Cannas et al., 1998). The two energy levels differed in terms of ingredients because the cereal grains used in the HE were substituted by beet pulps and soybean hulls.

Table 6 - Comparison of two levels of sugars and starch in TMR diets for East Friesian lactating ewes: characteristics of the diets (Cavani et al., 1990).

	<b>Sugar + Starch</b>	
	<b>15.7%</b>	<b>20.7%</b>
<b><i>Ingredients</i></b>		
Corn sil.+ beet pulp sil.+ meadow hay (% of DM)	64.9	64.9
Pelleted concentrate (% of DM)	19.1	35.1
Soy bean hulls (% of DM)	11.2	0
Distillers of cereals (% of DM)	4.8	0
<b><i>Composition</i></b>		
CP (% of DM)	13.9	13.6
Starch + sugars (% of DM)	15.7	20.7
ADF (% of DM)	34.4	28.5
NEL (Mcal/kg DM)	1.57	1.57

Table 7 - Comparison of two levels of sugars and starch in TMR diets for Friesian lactating ewes: main results (Cavani et al., 1990).

	<b>Sugar + Starch</b>	
	<b>15.7%</b>	<b>20.7%</b>
DIM at the beginning of the trial (d)	122	121
Length of the trial (d)	77	77
DMI (kg/d)	2610	2490
NEL intake (Mcal /d)	4.1	3.9
Initial BW (kg)	79.3	81.7
BW variations (g/d)	28.7 <sup>a</sup>	49.2 <sup>b</sup>
Milk yield (g/d)	1209 <sup>A</sup>	1107 <sup>B</sup>
6.5% fat corrected milk yield (g/d)	1186 <sup>A</sup>	1043 <sup>B</sup>
Milk fat content (%)	6.39 <sup>a</sup>	6.15 <sup>b</sup>
Milk fat yield (g/d)	75.8 <sup>A</sup>	65.8 <sup>B</sup>
Milk protein content (%)	4.86	4.94
Milk protein yield (g/d)	58.2 <sup>a</sup>	53.9 <sup>b</sup>

A, B: P< 0.01 a, b: P< 0.05



Table 8 - Composition of the diets of an experiment in which lactating Sarda ewes were fed diets differing in protein and energy concentration (Cannas et al., 1998)..

CP, % of DM	HE <sup>1</sup>				LE <sup>2</sup>			
	14.2	16.6	18.8	21.2	13.9	16.3	18.6	21.1
<b>Ingredients (% of DM)</b>								
Dehydrated alfalfa	30.2	30.2	30.2	30.2	30.0	33.4	36.6	40.0
Beet pulp	13.2	14.1	15.0	16.0	39.0	32.3	26.0	19.3
Soybean hulls	14.6	14.8	14.9	15.1	25.0	25.0	25.0	25.0
Corn grain	19.7	18.0	16.4	14.8	3.9	2.6	1.3	
Wheat grain	20.4	15.3	10.6	5.5				
Fish meal		0.7	1.3	2.0		0.7	1.3	2.0
Soybean meal		4.5	8.6	13.1		3.4	6.7	10.1
Corn gluten meal		0.7	1.3	2.0		0.7	1.3	2.0
Miner., vitam., ligands	1.9	1.7	1.6	1.4	2.2	1.9	1.7	1.5

<sup>1</sup> HE = high energy (1.65 Mcal/NE<sub>L</sub> kg DM).

<sup>2</sup> LE = low energy (1.55 Mcal/NE<sub>L</sub> kg DM).

The ewes fed the LE diets had, on average, higher DM intake but similar daily energy intake than the ewes fed the HE diets (Table 9), suggesting that fiber particle size did not limit DM intake. The ewes fed the LE diets had significantly higher milk yield and milk fat content compared to the ewes fed HE diets, while milk protein concentration did not differ between energy levels (Table 9). Milk yield was markedly affected by the protein concentration of the diets in both groups. For each CP level it was higher in LE than HE diets (Table 9 and Figure 17).

Another experiment was carried out during the 4th month of lactation on Sarda lactating ewes to compare diets with different NFC concentration (35% vs. 24%, DM basis) (Molle G., Cannas A., Bomboi G., unpublished). The diets were made up of 350 g/d of chopped dehydrated alfalfa and pellets ad libitum. The pellets differed in their ingredients (mostly because the cereal grains used in the NFC 35 diet were substituted by soybean hulls) and chemical composition (Table 10). The ewes fed the diets with the lowest NFC concentration (NFC 24) had higher DM intake but similar daily energy intake than the ewes fed the NFC 35 diets (Table 11).

Table 9 - Relationship between dietary crude protein and energy concentration and intake and milk production of Sarda sheep fed pelleted diets (Cannas et al., 1998).

Energy level <sup>1</sup>	Dietary Composition (% of DM)				Intake		Milk		
	CP	NFC <sup>2</sup>	NSC <sup>3</sup>	NDF	DM kg/d	NEL Mcal/d	Yield kg/d	Fat %	Protein %
HE	21.2	24.8	20.7	47.3	2.12	3.49	1.34	5.6	5.4
HE	18.8	27.4	24.1	48.0	2.33	3.84	1.34	5.4	5.3
HE	16.6	29.9	27.3	48.7	2.34	3.87	1.20	5.7	5.4
HE	14.2	32.5	30.7	49.4	2.02	3.35	1.16	5.7	5.7
LE	21.1	37.4	15.7	37.7	2.47	3.83	1.48	5.9	5.2
LE	18.6	38.9	15.2	37.9	2.54	3.94	1.50	5.7	5.3
LE	16.3	40.3	14.7	38.0	2.38	3.69	1.43	5.7	5.4
LE	13.9	41.8	14.3	38.1	2.18	3.37	1.26	6.0	5.5
HE Mean	17.7	28.7	25.7	48.4	2.20 <sup>m</sup>	3.64	1.26 <sup>M</sup>	5.58 <sup>M</sup>	5.45
LE Mean	17.5	39.6	15.0	37.9	2.39 <sup>n</sup>	3.71	1.42 <sup>N</sup>	5.82 <sup>N</sup>	5.36

<sup>m, n</sup> = P < 0.06    <sup>M, N</sup> = P < 0.005    <sup>1</sup> HE = High Energy, 1.65 Mcal of EN<sub>L</sub>/kg DM; LE = Low Energy, 1.55 Mcal of EN<sub>L</sub>/kg DM.    <sup>2</sup> NFC = non structural carbohydrates, calculated as: 100 - (NDF - NDF<sub>IP</sub>) - CP - EE - ash.    <sup>3</sup> NSC = starch and sugars.

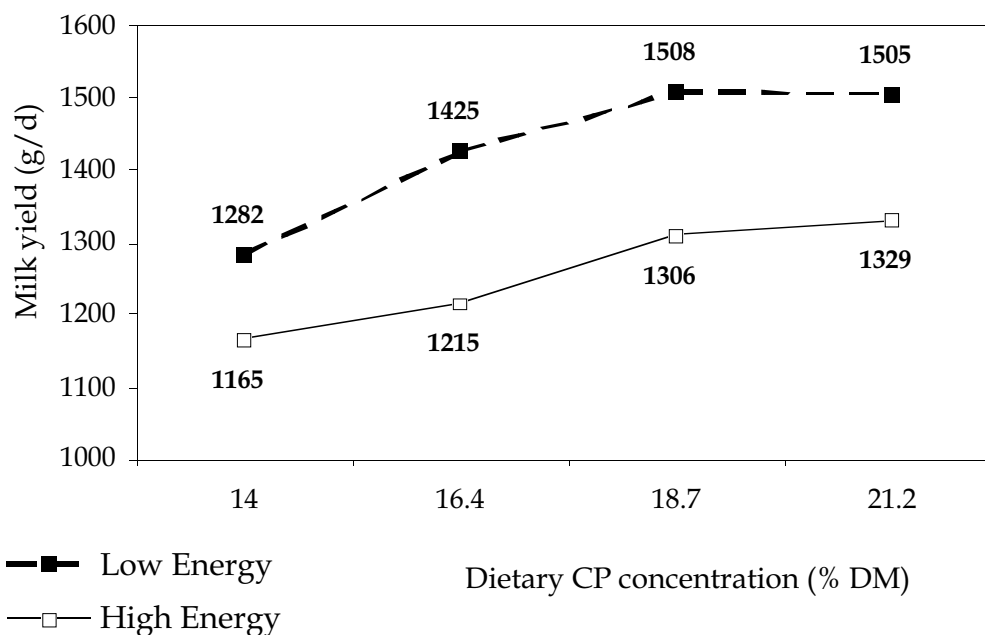


Figure 17 - Relationships between CP content of the diet and milk yield in two groups of housed lactating ewes fed either high energy (HE, 1.65 Mcal of EN<sub>L</sub>/kg DM) or low energy (LE, 1.55 Mcal of EN<sub>L</sub>/kg DM) pelleted diets. Milk yield in the preliminary period was 1.28 kg/d for the HE group and 1.22 kg/d for the LE group (Cannas et al., 1998).

This suggests that DMI was not limited by the high NDF content (51.2%) of NFC 24 diets, probably because of the small particle size of the fiber included in the pellets, but was regulated by requirements. The ewes fed the NFC 24 diet had significantly higher milk yield, lower milk protein content and similar milk fat content when compared to the ewes fed NFC 35 diets. BW variations and energy balance were slightly higher in the NFC 35 diet, even though these differences were not statistically significant.

Table 10 - Ingredients and chemical composition of two pellets differing in their NFC concentration and fed *ad libitum* to lactating Sarda ewes (Molle G., Cannas A., Bomboi G., unpublished).

	Pellet for diet NFC 35	Pellet for diet NFC 24
<b>Ingredients (% of DM)</b>		
Cracked corn grain	16.0	
Wheat grain	15.4	
Dehydrated alfalfa	29.5	29.5
Beet pulp	10.2	10.2
Soybean hulls	9.2	43.0
Soybean meal 44%	16.6	14.5
Minerals	0.8	0.8
Ligands	2.3	2.0
<b>Composition (% DM)</b>		
CP (% of DM)	20.2	19.5
NDF (% of DM)	36.7	51.2
NFC <sup>2</sup> (% of DM)	36.1	23.2
Starch (% of DM)	26.2	6.9

<sup>2</sup> NFC = non-structural carbohydrates, calculated as:  $100 - (\text{NDF} - \text{NDF}_{\text{IP}}) - \text{CP} - \text{EE} - \text{ash}$ .

In summary, these experiments suggest that during mid-late lactation the effects on milk of diets differing in their forage to concentrate ratio (and thus in their energy, NDF and NFC concentration) are not as clear as in the first part of the lactation. When the ewes are underfed, with shortage of energy, protein or both, the utilization of diets rich in concentrates improves milk yield. However, when the ewes are well fed, the utilization of large doses of concentrates rarely improves milk yield and often decreases it. It seems that when diets with different NFC concentration are compared, if the daily energy intake is similar between NFC levels, diets with low NFC concentration induce higher milk yield (experiments reported in the Tables 6-11). On the other hand, when the physical control of the diets keeps the intake of energy in low NFC diets lower than that of high NFC diets (such as in the experiment described in Table 4 and 5), milk yield is not affected by the utilization of diets rich in NFC, with energy partition favoring body reserve accumulation.

Practical reference values for dietary energy, NDF and NFC concentration for lactating ewes were reported by Cannas (2002b).

Table 11 - Main results of an experiment in which two pelleted feeds differing in their NFC concentration were fed *ad libitum* to lactating Sarda ewes together with 350 g/d of chopped dehydrated alfalfa (Molle G., Cannas A., Bomboi G.; unpublished).

	DIET		P<
	NFC 35	NFC 24	
BW (kg)	42.75	42.07	NS
DM intake <sup>1</sup>	2593	2943	0.005
NEL intake (Mcal/d)	4.21	4.25	NS
Milk yield (kg/d)	1825	2098	0.01
Milk fat (%)	5.06	5.19	NS
Milk protein (%)	4.75	4.45	0.003
Energy balance <sup>2</sup> (Mcal/d)	+1.683	+1.481	NS
BW variations (kg/d)	0.159	0.148	NS
Glucose (mg/dl)	62.1	59.8	0.07
Insulin ( $\mu$ U/ml)	14.6	22.6	0.002
Prolactin (ng/ml)	452	437	NS
Growth hormone (ng/ml)	3.36	5.20	0.02
Cortisol ( $\mu$ g/dl)	0.554	0.555	NS

<sup>1</sup> the DM intake of the dehydrated chopped alfalfa is included in the total, which was 284 g/d and 301 g/d for the NFC 35 and NFC 24 diets, respectively;

<sup>2</sup> NEL daily intake - energy requirements for maintenance and milk production.

### Interpretation of the experiments and nutritional implications

The experiments so far reported suggest that the effects of dietary forage to concentrate ratios (and thus dietary NDF and NFC) on milk yield and body reserve accumulation vary depending on the stage of lactation. During early lactation, large amounts of grains (NFC up to 35-40%) seem to help ewes with negative energy balance to reduce the deficit of energy and to produce more milk, while later on large amounts of grains (and thus of NFC) seem to be detrimental, because they stimulate fattening and not milk synthesis.

The reason for this difference is probably associated to variations of the hormonal status during lactation. The most important hormone implicated in the partitioning of nutrient between milk production and body reserve is the growth hormone (**GH**). When its blood concentration is high, dietary energy is used preferentially for milk production, while when it is low, body reserve accumulation is favored. During the first months of lactation, in ruminants GH concentration is usually high and insulin, which by itself stimulates uptake of glucose by peripheral tissues, is low. In addition, GH reduces the responsiveness of peripheral tissues to insulin (Rose and Obara, 1996). In this situation, there is a preferential utilization of glucose and lipogenic precursors by the mammary gland (Vernon, 1989). When lactation progresses, blood GH declines, insulin concentration increases and body fat tissues become very responsive to insulin action. This means that any increase in blood glucose stimulates the action of insulin, which favors glucose utilization for anabolic processes by peripheral tissues but not by the mammary gland, which is not responsive to insulin. The effect is a reduction of the glucose available for the mammary gland, with subsequent reduction of milk yield.

In sheep the action of insulin is probably even more important than in dairy cows, because blood GH concentrations are quite low for the whole lactation (Figure 18). This difference between sheep and dairy cows is the result of the high genetic selection that dairy cows have been subjected to. Genetically superior cows have higher and more persistent blood concentrations of GH than inferior cows (Peel and Bauman, 1987; Sorensen et al., 1998). Injection of GH in ruminants has positive effects on milk yield and lactation persistency. Dairy cows treated with GH behave as genetically superior cows do and tend to use the nutrients more for milk production than for body fat deposition (Peel and Bauman, 1987). GH injection in dairy cows increases milk yield by 10-25% and is more effective when used in mid-late lactation. In sheep GH injections are effective even in the first half of lactation (34% more milk between the 3<sup>rd</sup> and the 8<sup>th</sup> week of lactation; Fernandez et al., 1995b) and induce much stronger effects (Baldi, 1999) than in dairy cows, probably because of the lower natural GH blood levels of sheep. In some cases, the effects are really dramatic. For example, Assaf ewes in the third month of lactation increased their milk yield by 55% (from 1.91 to 2.97 liters/d), when compared to control ewes, after the injection of 0.1 mg/kg of BW of GH (Leibovich et al., 2001).

In summary, during the first part of the lactation the GH levels are high, insulin action is limited and the utilization of diets rich in concentrates increases blood glucose, which is preferentially used by the mammary gland for milk secretion.

In mid-late lactation, GH is low and insulin is very active. When diets rich in concentrates are used, propionate production in the rumen increases. Propionate is used by the liver to produce glucose, which in turn stimulates insulin action, with reduction of blood glucose available for mammary gland syntheses.

The beneficial effects of forage-rich diets in mid-late lactation can be explained by the fact that they stimulate rumen acetate production, which cannot be used to produce glucose and does not stimulate insulin action. In addition, forage-rich diets are eaten slowly, reducing the peaks of production of propionate and the insulin response (Takahashi et al., 1989). Acetate is both a metabolic fuel, and can in part substitute glucose in this, and a precursor of fat. Lactating ewes probably have a higher acetate requirement than lactating cows, because their milk has much higher fat to lactose ratio. Pethick and Lindsay (1982) found that acetate uptake by the lactating ewe udder represents a greater drain on acetate supply than the udder of dairy cows. In addition, in two of our experiments the diets with the lowest NFC concentration had significantly higher GH concentrations and lower glucose concentration (Tables 5 and 11). Indeed, hypoglycemia and underfeeding tend to increase blood GH concentrations (Breier, 1999; Hatfield et al., 1999). As discussed in paragraph 2, GH retards the involution of mammary secretory cells. Thus, the utilization of low NFC diets may affect nutrient partitioning positively and reduce involution of the mammary gland, as long as the energy intake is sufficiently high.

In practice, it is not easy to achieve high energy intake using diets low in NFC and rich in fiber. This can be done only if the fiber of the diet is highly digestible and does not impose physical constraints on intake. These physical constraints can be reduced by chopping or grinding the forage. Sheep can safely use diets with very low particle size, with positive effects on feed intake and milk yield (Rossi et al., 1991; Cannas, 1995; Cannas, 2002b). The high digestibility of fiber is a characteristic of young forages and of some feed by-products, such as soybean hulls and beet pulps.

The effect of forage quality on milk yield and persistency was evident when ryegrass and alfalfa pastures were compared (Figure 13; Bomboi G., Cannas A., Molle G.). Alfalfa typically has lower NDF concentration and higher fiber degradability than ryegrass; this often results in high intake and milk yield (Van Soest, 1994). Pasture management and characteristics affecting feed intake and milk yield in dairy ewes are discussed elsewhere (Cannas, 1996; Avondo and Lutri, 2002; Cannas, 2002b, Molle et al., 2002).

Soybean hulls and beet pulps are rich in highly digestible NDF and in pectins, which have high degradation rates but do not stimulate lactic bacteria (Van Soest, 1994). Indeed, the diets of the experiments in which high fiber intake induced high milk yield are all based on beet pulps and soybean hulls. Positive effects of soybean hulls on sheep milk yield were also reported by Zervas et al. (1998), in a trial in which Greek dairy ewes were fed 800 g/d of grass hay and 1200 g/d of three different concentrate formulations. One had 60% of ground corn as the main ingredient, in another corn was replaced by equal percentages of soybean hulls, in the third one the concentrate with soybean hulls included soybean oil as well. The latter treatment will be discussed in the section on fat supplementation. The ewes that used the concentrate with soybean hulls produced slightly more milk than the ewes fed corn-rich concentrate and had significantly higher milk fat concentration.

As a result, they produced significantly higher amounts of 6% fat corrected milk (936 vs. 806 g/d for soybean hulls concentrate vs. corn concentrate, respectively;  $P < 0.001$ ). The stage of lactation during which the experiment was carried out was not reported. However, the fairly low milk yield and the positive BW variations observed during the experiment suggest that it was carried out during mid-late lactation.

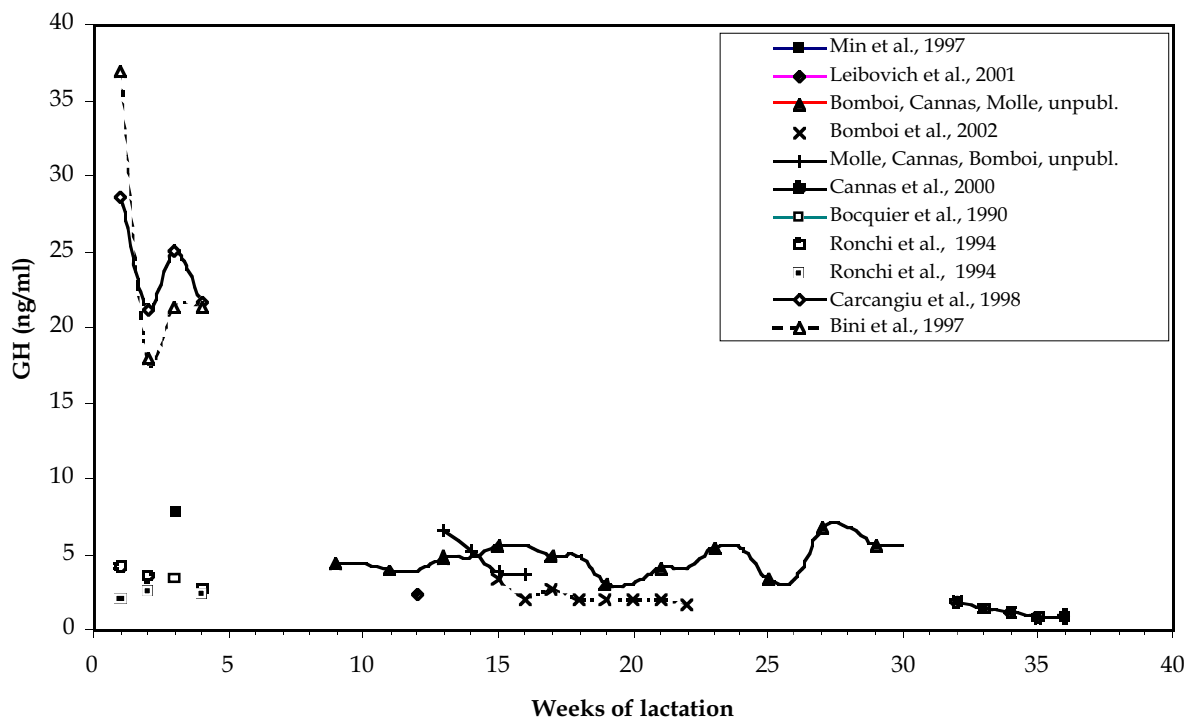


Figure 18 - Growth hormone concentration in lactating ewes during the lactation

## EFFECTS OF DIETARY FAT ON LACTATION PERSISTENCY

Dietary lipid supplementation has been used in diets for ruminants to increase dietary energy concentration and efficiency and, as a consequence, to increase milk yield in high producing animals. It has also been used to increase milk fat content and to modify the milk fatty acid profile. Here we will consider only the use of fat to increase milk yield and lactation persistency in sheep.

The effects of fat supplementation in sheep were examined by Chilliard and Bocquier (1993), Caja and Bocquier (2000) and Bocquier and Caja (2001). After reviewing the scientific literature, they concluded that fat supplementation increased milk yield in dairy cows but not in sheep and in goats and always increased milk fat content in sheep and goats but not always in dairy cows. No effects of calcium soaps of fatty acids (CSFA) on milk yield were also reported by McKusick et al. (1999b). However, these conclusions contrast with some other published experiments.

In lactating Sarda sheep, Rossi et al. (1991) compared complete pelleted diets with or without 4% CSFA. Dietary CP was close to 18% of DM. The ewes were fed hay and concentrates during a two-week preliminary period. They were then fed solely the experimental pelleted diets from the 10<sup>th</sup> to the 30<sup>th</sup> week of lactation. The utilization of the pelleted diets markedly increased milk yield in both groups, without differences between them until the end of the 6<sup>th</sup> experimental week (16<sup>th</sup> week of lactation) (Figure 19). After this, the milk yield of the two groups started to differ, with higher milk yield and lactation persistency in the ewes fed the pelleted diet added with CSFA. This difference was maintained until the end of the experiment. During the 20 experimental weeks the group with CSFA produced significantly more milk than the group without CSFA (188 kg vs. 168 kg). Milk fat content was also increased by the utilization of CSFA, but the effect was much faster than in the case of milk yield (Figure 20). Milk protein content was not affected by CSFA.

In another trial, Assaf ewes were fed diets with CSFA added (5.6% of dietary DM) from lambing to 90 days of lactation (Sklan, 1992). The main effects of CSFA addition were a significant increase in milk yield (1.36 vs. 1.59 kg/d for control and CSFA, respectively), with a concomitant increase in milk fat and reduction in milk protein compared to the control diet. The effects of CSFA were large until the end of the second month of lactation, then they quickly decreased.

Significant increases in milk yield and lactation persistency for the whole length of the experiment (from lambing to 140 days of lactation) were also observed by Laudadio et al. (2002) on Comisana ewes whose diet was supplemented with CSFA.

The positive effects of the addition of lipids were also observed when adding 5% soybean oil to the concentrates in the study of Zervas et al., (1998) previously reported when discussing soybean hulls effects. The addition of soybean oil significantly increased milk yield (947 g/d vs. 733 g/d) but significantly decreased milk fat and milk protein concentration compared to the same pelleted concentrate without oil. Similarly, Mele et al. (2002) added 4% soybean oil to diets differing in fiber concentration supplied to Sarda ewes in the third month of lactation. For both of the fiber levels studied, the addition of the unsaturated oil significantly increased milk yield. Milk fat concentration was not affected, while milk protein concentration decreased.

The lack of effects in diets supplemented with CSFA on milk yield and persistency reported in the reviews of Chilliard and Bocquier (1993), Caja and Bocquier (2000) and Bocquier and Caja (2001) contrast with the results of the experiments we reported. These differences are not easily explained. Possible reasons may be related to differences in intake of the pasture in grazing experiments, depressed diet digestibility and altered rumen metabolism in those experiments in which all the supplemented fat was added in high concentrations in the concentrates (and not diluted in the whole ration). Also, often the concentrates were supplied few times per day (usually twice per day), while in other cases they were added in total mixed rations.

## **DIETARY PROTEINS AND LACTATION PERSISTENCY**

Optimal dietary protein concentration should be calculated by dividing the required metabolizable protein (PDI or MP depending on the system used) by the predicted DM intake. However, when this approach is used the diets balanced for PDI or MP are often rather low in CP concentration, with values ranging between 11% and 15% CP (DM basis) (Cannas, 2000; Cannas, 2002a; Cannas, 2002b). These values are also lower than those reported for lactating dairy cows (NRC, 1988). Low dietary CP concentrations usually decrease intake, feed passage rate and milk yield (Van Soest, 1994; Cannas and Van Soest, 2000). Moreover, several experiments in which various dietary CP concentrations were tested in lactating ewes found that the highest milk yield was obtained with dietary CP of around 17%-19% (DM basis) both in early (Gonzalez et al., 1982; Gonzalez et al., 1984; Robinson, 1987a) and mid-late lactation (Pulina et al., 1990; Cannas et al., 1998). This suggests that in many cases sheep require more protein than predicted by most feeding systems. Optimal CP concentrations for lactating ewes were reported elsewhere (Cannas, 2002b).

In many of the experiments in which high protein concentration improved milk yield, the diets included rather large amounts of escape protein (feed protein not fermented in the rumen and digested in the intestine) because either protein sources of low degradability were used or high intake and feed rumen passage rates were achieved.

As an example, an experiment previously presented (Tables 8 and 9 and Figure 16), in which different dietary protein and energy concentrations were tested in Sarda ewes in mid lactation (Cannas et al., 1998) will be discussed here. The highest milk yield was obtained with dietary CP concentrations close to 19% for both energy levels considered. In this experiment, the diets included (Table 8) two protein sources of low degradability (fish meal and corn gluten meal) that were eaten at very high levels of intake (DM intake above 5% of BW). This probably induced high passage rates (and then high feed protein escape) of dietary protein.

Since lactating ewes usually have higher levels of intake than cows, the concentrations of dietary CP found in the experiments above mentioned imply that lactating ewes should have a higher daily intake of CP per kg of BW than lactating cows to meet their requirements. The high CP requirement per kg of BW and the high requirement in escape protein may be explained by the fact that sheep require high quantities of sulfur-containing amino acids for wool production (Bocquier et al., 1987). This suggests that methionine, the essential amino acid required in large amounts for wool production, and some co-associated essential amino acids (e.g. lysine) may be



limiting. In fact, the lack of any specific essential amino acid may result in serious dietary imbalances, wastage of proteins and decreased milk yield. Thus, the positive effect of diets rich in escape proteins on milk yield may be explained with their ability to provide essential amino acids to sheep.

To verify if methionine or other essential amino acids limit milk yield in sheep, we reviewed some of the publications in which protected forms of essential amino acids were supplemented to lactating ewes (Table 12). In the majority of the experiments the addition of methionine or lysine slightly increased milk yield but only in few cases the differences were significant (Table 12). Excess of dietary protein can have negative effects on milk yield and persistency of lactation, because the energetic cost of disposing the nitrogen in excess is very high (12 kcal/g of nitrogen; Tyrrel et al., 1972). This means that one hundred grams of CP in excess of requirements cost to the ewe the same energy required to produce 200 g of milk. Excess of CP intake is common in sheep grazing immature pastures, in which the CP concentration is often higher than 25-30% of DM. Thus, especially when energy availability is limiting, diets too rich in proteins can cause reduction of milk yield. In addition, excess of ammonia tend to increase nitrogen retention in the body at the expense of milk yield and milk protein yield (Malik et al., 1999). Therefore, the utilization of pastures or feeds with high soluble protein content can substantially reduce milk yield and lactation persistency.

Dietary protein unbalances can be fairly easily monitored by measuring milk urea concentration, which in sheep is a powerful nutritional indicator (Cannas et al., 1998; Cannas, 2002b).

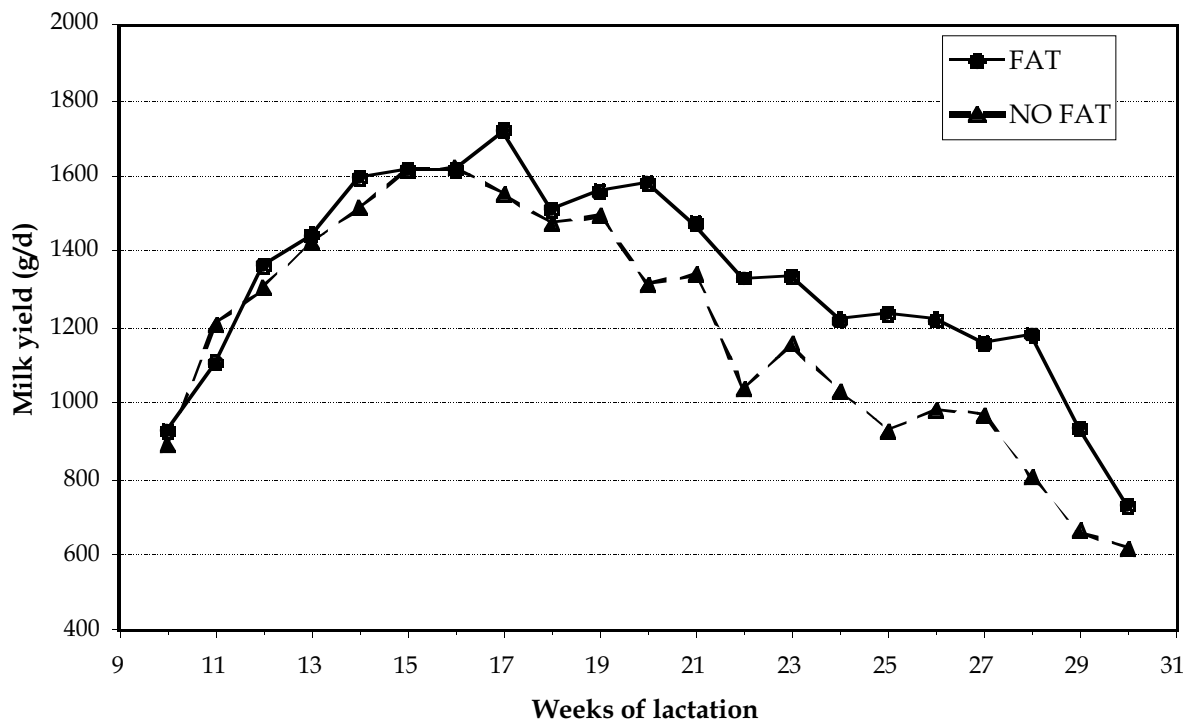


Figure 19 - Milk yield of Sarda ewes fed *ad libitum* pelleted diets supplemented with 4% of calcium salts of fatty acids (Rossi et al., 1991). • Diets supplemented; ▲ control.

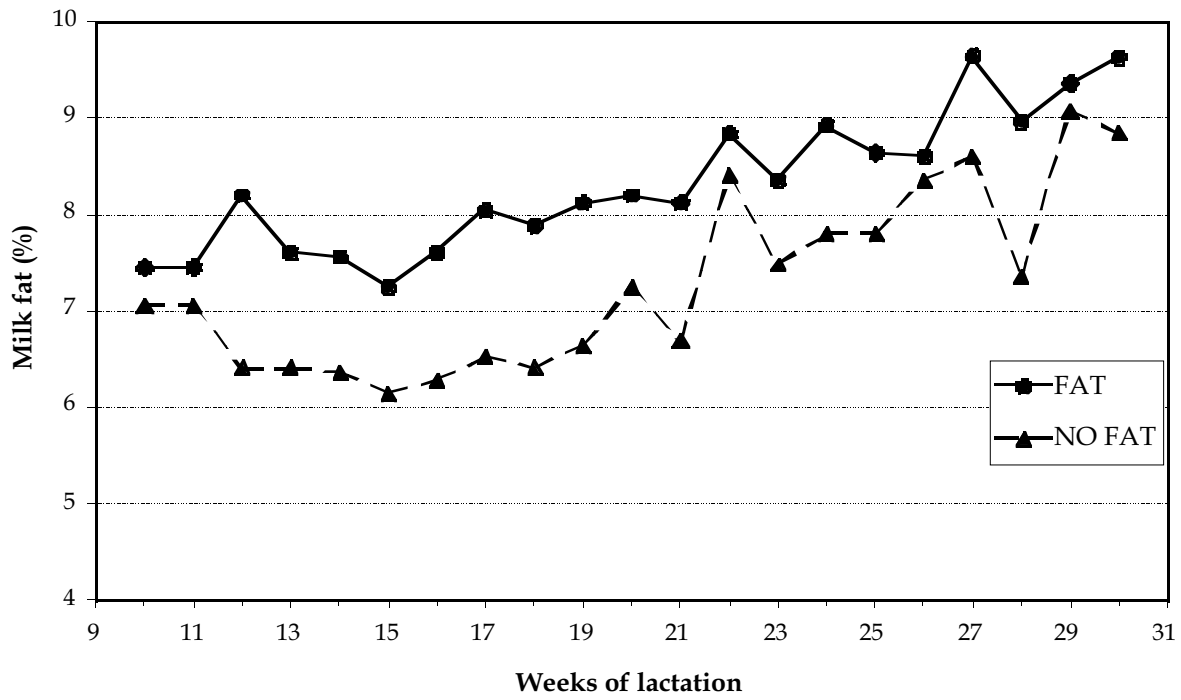


Figure 20 - Milk yield of Sarda ewes fed *ad libitum* pelleted diets supplemented with 4% of calcium salts of fatty acids (Rossi et al., 1991). • Diets supplemented; ▲ control.

Table 12 - Effects of supplementation of methionine and lysine on milk yield and protein content in ewes.

Author	Breed	Treatment daily doses	Milk yield g/d		Milk protein (%)		Month lactat.	Length trial (d)
			control	treated	control	treated		
Barry, 1980	Romney	2 g L Meth + 95g Na Caseinate	1884	2016 ns			1	42
Floris et al., 1988	Sarda	6 g (for 30d) + 12g (for 12d) Meth	706	760 ns	4.50	4.70 ns	3	42
Olivieri et al., 1986 and Supplizi et al., 1986	Sarda	2.4 g Meth		+ 288 **	5.97	5.80 ns	1	140
Lynch et al., 1991 <sup>1</sup>	Sarda	1.2 g Meth		+ 93 ns	5.97	6.09 **	1	140
Lynch et al., 1991 <sup>2</sup>	Black-face	3.80 g Meth + 3.97 g Lys - High CP diet	1900	2200 ns	5.46	5.60 <sup>3</sup>	1	49
Baldwin et al., 1993	Black-face	5.05 g Meth + 4.26 g Lys - Low CP diet	1780	1760 ns	4.83	5.23 <sup>3</sup>	1	49
Sevi et al., 1995	Dorset	2 g Meth	2110	2160 ns	4.74	4.60 ns	1	42
Sevi et al., 1995	Comisana	2.8 g DL Meth	644	665 ns	6.30	6.30 ns	2	112
Sevi et al., 1996	Comisana	5.6 g DL Meth	644	675 ns	6.30	6.40 ns	2	112
Sevi et al., 1996	Comisana	8.4 g Lys-HCl	644	774 *	6.29	6.14 ns	2	84
Chiofalo et al., 1996	Comisana	16.8 g Lys-HCl	644	613 ns	6.29	6.20 ns	2	84
	Comisana	4.9 g Lys + 1.4 g Meth	560	620 *	6.23	6.59 **	5	56

<sup>1</sup> The growth rate of the suckling lambs was higher in the treated vs. the control (509 vs. 433; P<0.05)

<sup>2</sup> The growth rate of the suckling lambs was higher in the treated vs. the control (382 vs. 458; P<0.05)

<sup>3</sup> no statistical tests were reported;

\* P<0.05 \*\* P<0.01

## CONCLUSIONS

Lactation persistency is controlled by a complex interaction of genetic, physiological and nutritional factors. For this reason, there are no simple and always successful nutritional strategies to improve lactation persistency. Milk yield in the second half of the lactation is markedly affected by the nutritional and non-nutritional events that occurred in the previous phases. Feeding techniques for the second half of the lactation should always keep in mind that sheep have been less selected for milk persistency than cows and tend to reduce milk yield rather easily when subjected to nutritional, but also environmental, stresses. Thus, the diet utilized and the nutritional status of the animals should be monitored by using nutritional indicators, such as BCS, milk urea or milk fat concentrations. The high variability in milk production among ewes of the same flock suggests that the animals should be divided in groups according to their level of production. If this is not done, it becomes very difficult to avoid excessive overfeeding of low producing animals and underfeeding of the ewes with the highest milk yield.

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