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Improving the quality and safety of sheep milk

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Abstract: In this chapter we review the factors that affect the quality of sheep milk and examine the strategies that farmers can adopt to improve the quantity and quality of milk produced by their sheep. Some of these factors, such as the genotype of the sheep, are difficult to control, but farmers have control over others, such as the nutrition and management of the milking sheep. To obtain high quality milk the sheep must be milked regularly and completely, which implies adopting appropriate milking and cleaning routines and milking equipment. It is also important that the sheep are healthy and that they receive adequate diets. Environmental contaminants that potentially make the milk unsuitable for processing should also be avoided. These factors and their influence on milk quality for the processing of milk into dairy products, especially cheese, are described and critically examined.

Key words: sheep milk, milk composition, milk quality, processing performance of milk, cheese.

13.1 Introduction: a historical perspective

Sheep and goats were domesticated before cattle, and people started to milk them soon after domestication (Clutton-Brock 1981, Anifantakis 1990). We know that sheep were milked and cheese was made from their milk since the Neolithic and the Bronze Age, as prehistoric milk boilers were found in Central Italy together with bones of sheep and goats (De Bellis 1982, Helmer and Vigne

2007), as well as direct evidence of milk residues in pottery found at archeological sites (Dudd and Evershed 1998). The ancient Greeks and Romans also milked sheep and produced cheeses from the milk. We know this from descriptions of cheese-making by Homer (*circa* 700 BC), Columella (*circa* 4 BC–65 AD) and Varro (36 BC). Sheep milking continued in Europe through the Middle Ages as reported by De Bellis (1982).

Sheep milking was practised traditionally in the Mediterranean countries, including Italy, France, Spain, Portugal, Greece, North Africa and some Asian countries. Milking occurred on a seasonal basis, often by keeping the flocks on the mountains in summer and shifting them to the plains in winter, to take advantage of the seasonality of pasture production in these areas. The consequence of this nomadic lifestyle was a low level of production and poor hygiene of the milk (Anifantakis 1990, Boyazoglu and Morand-Fehr 2001).

In these primitive farming conditions, selection towards specialized dairy breeds of sheep was never pushed to the same extent as in dairy cattle (Flamant and Morand-Fehr 1982). As sheep were reared for milking in very poor areas with low pasture production, breeders selected for sheep of low productivity and good adaptation to their environment, as often the available feed was not sufficient to sustain high milk production. It was only after the Second World War that many European governments started encouraging the settlement of shepherds, accompanying this with the selection of sheep for milk production. This was based on progeny testing of rams and resulted in large genetic gains, mainly in France (Flamant and Barillet 1982) and, to a lesser extent, in Spain, Greece and Italy (Bencini 1993, Lindsay and Skerrett 2003).

More recently countries that did not have a sheep milking tradition have started milking sheep mainly to satisfy the local demand from ethnic groups. Sheep are milked in Great Britain (Mills 1989), the USA (Thomas 2004), Australia and New Zealand (Bencini 1993). Here genetic gains have occurred by importing specialized breeds of dairy sheep, as progeny testing schemes are hindered by the small size of the industry and consequent lack of organization. This has also created two very different scenarios for the production and processing of sheep milk.

In this chapter we will first review the factors that affect the quality of sheep milk and then we will cover the strategies that can be adopted to improve sheep milk production and quality. As sheep milk is principally transformed into cheese, we will start by examining how the quality of sheep milk is of paramount importance for its processing into high quality cheeses.

13.2 Processing of sheep milk

Most of the sheep milk produced in the world is transformed into cheese. Some sheep milk yoghurt is produced in Greece, and fresh sheep milk is consumed rarely. Therefore the quality of sheep milk refers to its capability to be transformed into high quality dairy products, and to produce high yields of these

products from each litre of milk, often described as the processing performance of the milk.

For this reason, the quality of sheep milk is mainly evaluated in terms of its technological and coagulation properties, which are markedly affected by its concentration of fat and protein and somatic cell count (SCC). However, the quality of sheep milk is also affected by the presence and concentration of other components, including those with nutraceutical action such as conjugated linoleic acid (CLA) and other fatty acids (FAs), or those that affect the flavour of milk and its products. However, in all types of cheese, milk protein contributes more than milk fat to total cheese yield (Pulina *et al.* 2006a).

The clotting properties of milk are affected by the composition of the milk (Anifantakis 1986, 1990, Park *et al.* 2007), by the microbiological quality of the milk, by its somatic cell count (Pulina 1990, Kalantzopoulos 1994) and by the cheese-making process itself (Alais 1984).

Cheese makers have some control over the clotting conditions, and can vary pH to achieve the desired acidity by varying the percentage of inoculum of starter cultures (Cogan and Hill 1993). They can also standardize the fat content (Kalantzopoulos 1993) or increase the amount of soluble calcium in milk by adding calcium chloride (Fox 1993). This is often done with milk that does not clot (Alais 1984). Cheese makers also control the temperature and the rennet concentration at which the clotting takes place. However, cheese makers have little or no control over the composition and quality of the milk that reaches the cheese factory. This should be of high microbiological quality, free of antibiotics and have a composition within acceptable limits, but this does not always occur, despite the fact that often the payment for the milk is based on its quality (Pulina 1990).

High protein, fat and total solids concentrations in the milk are associated with high yields in the resulting dairy products. As a consequence, the milk of sheep has a higher yield of dairy products than the milk of cows and goats because it has higher concentrations of protein, fat and total solids (Anifantakis 1986, 1990).

Therefore factors that affect the composition of the milk will affect the yield and quality of dairy products obtained from the milk.

13.3 Factors affecting the quality of sheep milk

As discussed above, the cheese maker at the cheese factory receives milk of a certain composition and can do very little to change it. The farmer, by contrast, has some control over the environmental factors that affect the quality of sheep milk. These are discussed below.

13.3.1 Genetic factors

The breed and genotype of sheep can affect the quality of the milk produced. Selection for dairy production has led to the creation of dairy breeds of sheep that produce more milk than meat and wool sheep. For instance, the Awassi

dairy type can produce up to 1000 litres of milk per lactation (Epstein 1985), but the Poll Dorset, a meat breed, produces only 100–150 litres of milk per lactation (Geenty and Davison 1982).

There is a negative correlation between milk yield and milk composition, so that when animals produce more, the milk usually has a lower concentration of fat and protein (Bencini and Pulina 1997, Table 13.1). This relationship applies not only to the more productive breeds when compared with the less productive (Flamant and Morand-Fehr 1982, Casu and Sanna 1990), but also, within a flock, to those animals that produce more milk (Barillet *et al.* 1986), and within the same animal producing at different levels throughout its lactation (Casoli *et al.* 1989, Pulina 1990). This is generally attributed to the fact that milk volume is determined by lactose secretion, and in highly productive dairy animals the synthesis of fat and protein does not keep up with that of lactose when high rates of milk secretion are achieved (Bencini and Pulina 1997, Pulina *et al.* 2006a). As a consequence, with high milk production, the total amount of cheese produced from the milk can be higher, but the relative yield of cheese from each litre of milk will be lower.

Table 13.1 Concentrations (g/100 g) of protein and fat in different breeds of sheep

Breed	Protein	Fat	Source
Awassi	6.05	6.70	Mavrogenis and Louca (1980)
Boutsiko	6.04	7.68	Voutsinas <i>et al.</i> (1988)
Bulgaria population	5.83	8.10	Baltadjieva <i>et al.</i> (1982)
Chios	6.00	6.60	Mavrogenis and Louca (1980)
Clun Forest	5.90	5.80	Poulton and Ashton (1970)
Comisana	7.30	9.10	Muscio <i>et al.</i> (1987)
Dorset	6.50	6.10	Sakul and Boyland (1992)
East Friesian	6.21	6.64	Shalichev and Tanev (1967)
East Friesian cross	4.98	5.00	Thomas <i>et al.</i> (1998)
Egyptian population	5.84	8.30	Askar <i>et al.</i> (1984)
Epirus	6.56	7.85	Simos <i>et al.</i> (1996)
Fat-tailed	6.40	6.26	Mavrogenis and Louca (1980)
Finn	5.40	6.00	Sakul and Boyland (1992)
Karagouniki	6.60	8.70	Anifantakis (1986)
Karakul	5.57	7.36	Kirichenko and Popov (1974)
Lacaune	5.81	7.14	Delacroix-Buchet <i>et al.</i> (1994)
Massese	5.48	6.79	Casoli <i>et al.</i> (1989)
Merino	4.85	8.48	Bencini and Purvis (1990)
New Zealand Romney	5.50	5.30	Barnicoat (1952)
Rambouillet	5.90	6.10	Sakul and Boyland (1992)
Romanov	6.10	5.90	Sakul and Boyland (1992)
Sarda	5.89	6.61	ARA (1995)
Suffolk	5.80	6.60	Sakul and Boyland (1992)
Sumava	6.47	7.93	Flam <i>et al.</i> (1970)
Targhee	4.51	9.05	Reynolds and Brown (1991)
Tzigai	5.45	7.41	Margetin (1994)
Vlachiki	6.52	9.05	Anifantakis (1986)
Welsh Mountain	5.40	6.20	Owen (1957)

The genotype of the sheep may also affect the clotting properties of the milk through different genetic variants of the casein fractions (McLean *et al.* 1984, 1987). For dairy cows, the presence of certain casein genotypes can affect the composition of the milk (Aleandri *et al.* 1990), and Italian researchers have identified individual cows carrying particular genetic variants of the κ -casein which make the milk unsuitable for Parmesan cheese making due to poor coagulation (Losi and Mariani 1984).

In European dairy sheep, polymorphism of the casein has been reported by Lyster (1972), Arave *et al.* (1973), Davoli *et al.* (1985), Russo *et al.* (1981), Chiofalo *et al.* (1982), Bolla *et al.* (1985, 1986) and Manfredini *et al.* (1987). The α_{s1} casein variant named 'Welsh', the frequency of which varies from 2.2% (Chiofalo and Micari 1987) to 22% (Caroli *et al.* 1989), causes a reduction in casein content and a worsening of milk clotting properties in homozygous animals and, to a lesser extent, in heterozygous animals (Piredda *et al.* 1993). In Australian sheep, Thomas *et al.* (1989) and Clément *et al.* (2006) reported the existence of genetic variants of the casein, but it is still not clear whether these genetic variants have an effect on clotting properties and cheese outcome.

Few studies have been conducted on renneting times, coagulation patterns, rates of curd formation and curd firmness of sheep milk (Askar *et al.* 1984, Manfredini *et al.* 1987, Casoli *et al.* 1992, Delacroix-Buchet *et al.* 1994, Bencini and Johnston 1996, Bencini 2002). All these studies confirmed that sheep milk has short renneting times, fast rates of curd formation and high consistency of the curd, characteristics that are all associated with high yields of cheese from each litre of milk.

Despite the reported differences in composition of the milk between different breeds of sheep, Italian researchers have shown that there was no difference in cheese-making performance of milk between the breeds Sarda, Comisana, Massese and Delle Langhe (Chiofalo *et al.* 1989, Casoli *et al.* 1990, Ubertaine *et al.* 1991).

In France, Delacroix-Buchet *et al.* (1994) calculated the coefficients of genetic repeatability in the Lacaune breed for renneting time (0.57), rate of curd formation (0.48) and curd consistency (0.53) and concluded that, since these repeatability values are similar to the repeatability for milk production (*circa* 0.5), family type selection would be needed to achieve genetic progress.

13.3.2 Somatic cell count

The health of the sheep, and especially that of the mammary glands, affects the quantity and the quality of the milk produced. Mastitis is the most common pathology of the mammary gland in sheep dairies and is economically important because it reduces milk production and causes qualitative changes in milk composition, which alter the processing performance of the milk and the qualitative characteristics of the dairy products derived from it. This is due to a decreased synthetic capacity of the mammary secretory cells and to an increased permeability of the mammary epithelium that cause the passage of blood com-

ponents directly into the milk and an increase in the somatic cell count of the milk (Morgante *et al.* 1994). As a consequence, the milk of dairy sheep suffering from mastitis does not clot and is not suitable for cheese production (Casoli *et al.* 1992).

13.3.3 Microbial cell count

The microbial cell count in milk is due to the presence of microorganisms of which some (*Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp.) can be advantageous for transformation into cheeses, while others can cause human diseases (e.g. *Listeria*, *Salmonella*, *Brucella*) or problems in the maturation of the dairy products (e.g. Enterobacteriaceae, coliforms, psychrotrophs, *Clostridium* spp.; Fatichenti and Farris 1973).

Psychrotrophic bacteria such as *Pseudomonas*, *Leuconostoc* and *Micrococcus* thrive at temperatures below 7°C and produce lipolytic and proteolytic enzymes which destabilize the casein micelles and alter the clotting properties of milk (Nuñez *et al.* 1989, Uceda *et al.* 1994a, 1994b).

Enterobacteriaceae and coliforms are generally of faecal origin and ferment the lactose in the cheeses, producing large quantities of gas, causing early spoilage of the cheese (Gaya *et al.* 1987). The occurrence of undesirable bacteria can be avoided by applying correct milking and milk handling procedures.

Health regulations often prescribe the maximum bacterial and somatic cell counts allowable for the processing of sheep milk. These regulations vary for different countries and in some countries all dairy products must be produced from pasteurized milk. This is not strictly required in the Mediterranean countries, where some cheeses are produced from raw milk.

13.4 Physiological factors affecting the quality of sheep milk

13.4.1 Age and parity

Maiden ewes produce less milk than older ewes and maximum yields are generally achieved at the third or fourth lactation, after which total lactation yields tend to decrease (Bencini and Pulina 1997). As these order-of-parity factors are often confounded with the age of the ewes, it is impossible to distinguish between the two. The parity of the ewes affects milk composition: with increasing lactation number the milk contains higher concentrations of fat and protein, higher somatic cell counts and lower concentrations of lactose (Bencini and Pulina 1997).

Since the negative relationship between yield and milk quality has been confirmed to apply to individual animals within a flock (Barillet *et al.* 1986), changes in milk yield brought about by age and lactation number are likely to result in changes in the composition of the milk as reported by Pulina (1990) and Pulina *et al.* (1992, 2006a).

13.4.2 Stage of lactation

The stage of lactation markedly affects the amount of milk produced. Lactation begins at parturition and daily yields increase rapidly for the first few weeks. Peak yields are achieved around the third to fifth week of lactation (Bencini and Pulina 1997). After the peak, lactation declines more or less rapidly depending on the breed and genotype and on individual dairy potential. The concentrations of fat, protein (both casein and whey protein), total solids and somatic cells are high at the beginning and end of lactation, and low at peak lactation, while the concentration of lactose follows closely the lactation yield (Bencini and Pulina 1997). The mineral content of milk is also affected by the stage of lactation: throughout lactation there is an increase in chloride (Pauselli *et al.* 1992) and magnesium and a reduction in potassium (Polychroniadou and Vafopoulou 1984).

The processing performance of the milk also changes with the stage of lactation. As lactation progresses the clotting properties of the milk tend to worsen, with an increase in renneting time and rate of curd formation and a decrease in the consistency of the curd (Ubertalle 1989, 1990).

13.4.3 Weight of the ewes

Most authors agree that heavier ewes produce more milk (Burris and Baugus 1955, Owen 1957, Boyazoglu 1963); however, there are few reports on the effect of liveweight on the quality of the milk for cheese making. Pulina *et al.* (1994a) found positive phenotypic correlations (from 0.26 to 0.56) between the liveweight of Sarda ewes and the concentrations of fat and protein of their milk for the first 10 weeks of lactation.

13.4.4 Number of lambs born or weaned

Sheep suckling twin lambs produce more milk than those suckling single lambs, and ewes rearing triplets produce more milk than those rearing twins (Bencini and Pulina 1997). However, there are few and contradictory reports on the effect of number of lambs reared or born on the quality of milk. Gardner and Hogue (1964) reported that Rambouillet and Columbia ewes rearing single lambs had a lower concentration of fat in their milk than ewes rearing twins. By contrast, in a later study, these authors reported that Hampshire and Corriedale ewes that gave birth to single lambs produced milk with a higher concentration of fat and protein (Gardner and Hogue 1966). This was also reported by Serra *et al.* (1993) who observed that Sarda ewes that gave birth to single lambs produced milk with a higher concentration of fat and protein throughout lactation, but their milk production was lower than that of sheep with twin lambs. The negative relationship between yield and quality of milk may explain why twin-bearing ewes that produce more milk have lower concentrations of fat and protein in the milk. The contrasting result reported by Gardner and Hogue (1964) may be due to the small number of ewes (10 single- and 10 twin-bearing ewes) used in their experiment.

Milk yield is greater in ewes bearing multiple lambs even if they do not nurse them (Pollot and Gootwine 2004) but if dairy ewes are allowed to suckle their lambs for the first few weeks of lactation the yields of milk from ewes that reared twin lambs should be, at least initially, higher than those of ewes rearing singles. For this reason in the Middle East the ewes are allowed to suckle their lambs after the evening milking (Folman *et al.* 1966, Morag *et al.* 1970, Eyal *et al.* 1978). This weaning method affects the composition of the milk because sheep that are nursing their lambs do not have a normal milk ejection reflex (Bencini 1999, Marnet and McKusick 2001, McKusick *et al.* 2002) and some sheep milk producers advocate the destruction of newborn lambs to simplify the management of the milking operations. However, sheep that are allowed to feed their lambs produce more milk and have longer and more persistent lactations, resulting in a greater total lactation yield (Bencini 1999). In support of this, Ubertalle (1990) reported that early weaning of the lambs worsened the consistency of the curd derived from the milk even though the composition of the milk was not affected. This is probably due to the fact that oxytocin and prolactin that normally prevent mammary involution are reduced if lambs are removed early (Turner and Huynh 1991) and their reduction results in a reduction of mammary DNA and an increase of plasminogen activators. These convert plasminogen into plasmin, which is involved in the hydrolysis of β -casein, so that the final consistency of the curd is reduced.

13.5 Management factors affecting the quality of sheep milk

13.5.1 Milking techniques

In the Mediterranean countries sheep are occasionally still hand-milked with the consequence of poor hygiene and high bacterial counts in the milk (Anifantakis 1990). However, the concentration of protein and fat in the milk does not differ between hand and machine-milked ewes (Casu *et al.* 1978a).

In countries where sheep milking has been introduced recently, sheep are milked by machine and the milk has better microbiological characteristics. The machine-milking of sheep has been reviewed by Purroy Unanua (1986). The design of milking parlours and the organization of the milking operations have been described by Enne (1976) and Pazzona (1980) in Italian and by Kervina *et al.* (1981), Mills (1989) and Stubbs *et al.* (2009) in English. The practical recommendations of Stubbs *et al.* (2009) are particularly relevant to the emerging sheep-milking industries of Australia, New Zealand and, to a lesser extent, the USA.

13.5.2 Milking interval and frequency

As the rate of milk secretion is controlled locally by the feedback inhibitor of lactation (FIL), a fraction of the whey proteins present in milk (Wilde and Peaker 1990, Wilde *et al.* 1996), the interval and frequency of milking assume

paramount importance in affecting the yield of milk. The findings of Denamur and Martinet (1961) and Grigorov and Shalichev (1962) provided some indirect evidence that an autocrine control of milk secretion is present in sheep. This was later confirmed by Bencini *et al.* (2003). Therefore, the interval between milking, the milking frequency and the adoption of stripping methods to remove additional milk and ensure completeness of milking increase both the daily output of milk and the total lactation yield of dairy ewes by removing the inhibitory effect of milk accumulated in the alveolar tissue of the mammary glands.

In dairy sheep, reducing the frequency of milking results in a loss in milk production (Morag 1968, Karam *et al.* 1971, Bencini *et al.* 2003), but there are few and contrasting reports on the effect of milking frequency and interval on the composition of the milk. Casu and Labussière (1972) and Huidobro (1989) reported that omitting one or both of the milkings on a particular day of the week did not affect the composition of the milk. When the milking frequency was reduced from twice to once daily the composition of the milk was not affected in the studies of Casu and Boyazoglu (1974), De Maria-Ghionna *et al.* (1982) and Cannas *et al.* (1991), but fat and protein concentrations were increased in the studies of Battaglini and De Maria (1977) and Battaglini *et al.* (1977, 1979), and reduced in a study by Morag (1968). When the milking frequency was increased from two to three times daily, milk composition was not affected according to Morag (1968) and Cannas *et al.* (1991), but the concentrations of fat and protein were increased according to Mikus and Masar (1978).

Such contrasting reports may be due to the fact that these studies were conducted with different breeds of sheep, which would have efficient or inefficient autocrine control of milk secretion according to the degree of selection for dairy production. Bencini *et al.* (2003) reported that milk composition was affected by the milking interval and frequency in breeds that are not selected for dairy production because they have an efficient control of milk secretion. On the contrary, breeds selected for dairying have an impaired autocrine control mechanism and therefore do not respond as much to changes in milking frequency (Nudda *et al.* 2002).

13.5.3 Stripping method

Labussière (1988) showed that European sheep had two different kinds of milk ejection patterns: some ewes released their milk in a single peak and others had a delayed let-down, so that two distinct peaks were observed in the milk release curve. Double-peaked sheep are present in a greater proportion within the specialized dairy breeds. Labussière *et al.* (1969) studied the milk ejection reflex in Préalpes ewes and concluded that the second peak of milk ejection occurred because the ewes released oxytocin, which resulted in milk ejection. Labussière (1969) and Ricordeau (1974) observed that the milk obtained from double-peaked animals had a higher fat concentration and concluded that the double peak was due to a delayed release of endogenous oxytocin, so that the first peak

corresponded to the removal of the cisternal milk and the second peak, within a minute of the first, corresponded to the alveolar milk ejected due to the release of endogenous oxytocin. This is in agreement with Ranieri (1993) who measured the composition of milk fractions collected during milking and observed a progressive increase in the concentration of fat, but no changes in the concentrations of protein and lactose as milking proceeded. Mayer (1990) measured plasma oxytocin concentrations in single- and double-peaked ewes and confirmed the conclusions of Labussière (1988) and Purroy Unanua (1986), that ewes with a double peak had an effective ejection reflex, which allowed for a better removal of milk from the mammary glands. By contrast, ewes with a single peak of milk ejection did not release oxytocin so that only the cisternal milk, which has a low concentration of fat, was collected.

The fact that animals with single peaks have high residual milk, shorter lactations (Labussière 1985, 1988, Purroy Unanua 1986) and lower concentration of fat in the milk (Mikus 1970) supports the adoption of practices to increase the removal of milk from the mammary glands. Labussière (1985, 1988) and Bencini and Knight (1994) demonstrated that stripping by hand or by machine is necessary for unselected sheep that have only one peak of milk ejection.

However, for Sarda sheep with a double peak of ejection, Casu *et al.* (1978b) reported that double cupping can be eliminated without affecting the composition of the milk, and Purroy Unanua (1986) reported that manual stripping can be replaced successfully with machine stripping, or double cupping, without compromising the amount of milk withdrawn. Additionally, McKusick *et al.* (2003) found that when machine stripping was eliminated in highly selected East Friesian sheep the 14% loss in milk yield was compensated by a greater throughput of sheep.

13.5.4 Environmental conditions and milk composition

Air temperature is a key microclimatic parameter for the well-being of sheep and is affected by airflow and humidity. Exposure of sheep to temperatures above 30°C depressed the immune response and decreased milk production as well the casein and fat concentration of the milk, and worsened the hygiene and quality of milk with an increase in neutrophiles, staphylococci, coliforms and Pseudomonadaceae in the milk. This was accompanied by a worsening of the processing performance of the milk, due in part to a lower concentration of calcium, phosphorus and casein, and in part to the increased activity of plasmin, which is the main proteolytic endogenous enzyme in milk (Sevi *et al.* 2001a),

Air quality is also important for sheep housed indoors. Carbon dioxide, ammonia, sulphide and methane are the main gases detected in increased levels in animal houses. A low ventilation regimen (33 m³/h per ewe) caused a significant reduction in milk yield, depressed immune reactivity, and increased plasma cortisol in ewes during summer. While casein concentration was lower in the milk of the sheep kept under the low ventilation regime, clotting time, curd firmness, pH and SCC of milk were unaffected (Sevi *et al.* 2001b).

Sevi *et al.* (2001b) also found that total microbial count and coliforms were lower in the air if sheep had 2 m² of space each compared to sheep that had only 1.5 m² or 1.0 m². The sheep also produced more milk in the less crowded environment, and the milk had a greater concentration of protein, casein and fat.

13.5.5 Management of the ewes

Some management practices such as shearing, out-of-season breeding, artificial lighting/photoperiod and the use of hormones may affect the quantity and quality of milk produced.

Shearing

Shearing sheep before or immediately after lambing increases the concentration of protein and fat in the milk of Poll Dorset ewes (Knight *et al.* 1993). This may be caused by an increase in feed intake (Wodzicka-Tomaszewska 1964, Vipond *et al.* 1987) leading to an increase in blood glucose (Kirk *et al.* 1984), probably in response to cold stress (Wheeler *et al.* 1962).

Rassu *et al.* (2009) found that milk fat content increased from 6.37% pre-shearing to 6.94% post-shearing. The content of fatty acids C8, C10, C12 and C16 also significantly increased from pre-shearing to post-shearing but without affecting the content of long-chain fatty acids (C > 18:0). This suggests that the effects of shearing on milk composition are not simply due to temperature and that the mechanisms involved in the modification of the FA profile of milk after shearing should be elucidated.

Breeding out of season

In the Mediterranean countries, sheep milking is seasonal, and even the largest and most modern sheep milk cheese factories are closed during summer.

Sevi *et al.* (2004) observed that the milk yielded by winter-lambing ewes had lower fat and protein contents compared to that of autumn-lambing ewes, as well as worse processing performance and hygienic characteristics. Irrespective of the lambing season, a higher SCC was recorded in late, compared to early and mid-lactation milk (6.16 vs. 5.93 and 5.87 log₁₀ somatic cells/ml) as well as a marked worsening in clotting properties. Reduced cheese-making efficiency was associated with a reduction in the casein and fat content of milk from autumn-lambing ewes, and decreased calcium and phosphorus content in the milk of ewes that lambed in winter. In Europe, sheep milk produced in summer is strongly affected by the combined effects of seasonal changes in climate and herbage availability and variations in the metabolic status of the sheep with the advancement of lactation. For these reasons, the milk has poor cheese-making performance (Delacroix-Buchet *et al.* 1994) due to long renneting times, poor consistency of the curd and high proteolytic and lipolytic activities.

Jaeggi *et al.* (2005) observed a decrease in total solids in the milk collected in winter, late spring and summer as the season progressed and attributed this to the hot weather and poorer quality pastures grazed by the animals in summer. As a

result, cheese yield was higher for milk collected in winter than for milk collected in spring and summer. Thompson *et al.* (1982) reported that high temperatures do not have an effect on the composition of sheep milk, but long day lengths result in lower protein concentrations in the milk (Bocquier *et al.* 1990) and in reduced rates of secretion of fat and protein (Pulina *et al.* 1994b). In summer, strategies to reduce the impact of high temperatures on lactating ewes and supplementation with minerals are necessary to sustain the yield and quality of milk and cheese (Sevi *et al.* 2004).

The high variability of milk quality in summer may be attributed to the poor nutritive value of pasture, as Pulina *et al.* (1993) demonstrated that a balanced ration restored the cheese-making performance of summer milk. In order to improve the efficiency of cheese factories and exploit the greater market demand during the summer period, Piras *et al.* (2007) proposed a lambing plan to produce milk also in summer and early autumn, using irrigated pastures, resulting in yields and quality of milk that were very comparable to milk produced in the traditional system.

When sheep dairying was first established in Australia and New Zealand, most farmers opted for a year-round supply of milk, which was made possible by the fact that the local sheep were less seasonal than the European breeds, and if appropriately managed, could be bred out of season (Scaramuzzi and Martin 1984, Signoret 1990). However, sheep dairy farmers experience a number of problems connected to the out-of-season breeding of their dairy flocks. First, only a proportion of ewes will mate out of season, so most of the lambings are concentrated in winter. Second, the availability of good pasture during summer is low, especially in Australia, and those sheep that have lambed tend to produce less milk. As a consequence severe shortages of milk occur during the summer months, when the demand for fresh sheep milk yoghurt is highest. The differential nutrition also changes the processing performance of the milk and consequently the quality of the derived dairy products. So, it is difficult to maintain a constant and consistent production throughout the year. As breeding out of season is often pursued through the use of hormones, it is also not consistent with the recent trend towards clean, green and ethical animal production that aims at minimizing the use of hormones (Martin *et al.* 2004).

Artificial lighting/photoperiod

Photoperiod determines the reproductive season in sheep, especially at high latitudes, but it also affects milk production and milk composition. Exposing the sheep to long days increased milk yield but resulted in lower concentrations of fat and protein in the milk (Bocquier *et al.* 1990, Pulina *et al.* 1994b, Chilliard and Bocquier 2000). By contrast, Morrissey *et al.* (2008) found no difference in the composition of the milk. Short day lengths before lambing had a positive effect on milk production in the following lactation, possibly because photoperiod affects progesterone, prolactin and placental lactogen that regulate mammary development during pregnancy (Pollot and Gootwine 2004).

Use of hormones

Growth hormone increases milk production in dairy cows (Bauman 1999). This occurs also in dairy sheep, where the increase in milk production is accompanied by increases in milk fat concentration and reductions in milk protein, which is not desirable for cheese making (Fleet *et al.* 1986, 1988, Holcombe *et al.* 1988, Pell *et al.* 1989, Sandles *et al.* 1988, Stelwagen *et al.* 1993). In any case, the use of hormones to increase milk production is not allowed in Europe and Australia.

13.5.6 Nutrition

Since nutrition affects both the yield and the composition of the milk produced, it also affects the quantity and quality of cheese (Bencini and Pulina 1997). Several nutritional factors affect milk fat concentration and yield. Among the most important factors are the energy balance (EB) of the ewes, the concentration, intake and source of dietary neutral detergent fibre (NDF) and non-fibrous carbohydrates (NFC), the particle size of feeds and fibre, as well as the amount, physical characteristics and fatty acid composition of dietary fat supplements. Interactions among these factors and other less important ones make prediction of milk fat concentration complex. These will be examined in more detail in the next section, on the improvement of sheep milk.

13.6 Improving sheep milk production

13.6.1 A genetic approach

The extension of dairy sheep breeding goals to milk quality and functional traits should be carefully evaluated both from a technical and economical viewpoint. The classical quantitative selection approach for improving milk composition is difficult due to the negative genetic correlation with milk yield and the high cost of phenotype recording. A selective goal should be to increase the fat and protein yield independently from milk yield to avoid any negative consequences on the fat and protein concentrations (Casu *et al.* 2009).

Aggregate genetic indexes that also consider milk fat and protein contents have been adopted in France for the Lacaune sheep, in order to prevent a possible reduction in milk fat and protein contents caused by selection based on milk yield (Barillet 1997). As almost all sheep milk is processed into cheese, Othmane *et al.* (2002) suggested the use of individual cheese yield that can be recorded on each test day. This new variable, which is correlated to the milk fat and protein contents ($r = 0.52$ and 0.38 , respectively), has the advantage of expressing in a single term the suitability of milk for cheese processing.

The increased knowledge on genomic regions controlling quantitative traits of economic interest could be implemented into marker assisted selection schemes for improving breeding value estimation, enhancing selection intensity and reducing generation interval (Cappio-Borlino *et al.* 1996, Russo and Fontanesi 2001). Research into quantitative trait loci (QTLs) is constrained by

the extensive time and resource requirements, mainly due to the large number of animals that have to be genotyped to obtain a reasonable statistical power of the experimental design (Rebai *et al.* 1995, Carta and Elsen 1999). However, research has been carried out on QTL mapping in dairy sheep and interesting results for milk composition have been reported (Table 13.2).

On the other hand, selection for sheep milk quality has to deal with the continuous evolution of the concept of quality, which includes a marked drift toward nutraceutical aspects. A significant example is represented by the recent interest in the milk content of conjugated linoleic acid (CLA). Differences in the genomic region containing the stearoyl Co-A (SCD) gene, which encodes for the delta-9-desaturase enzyme, could affect the CLA content of the milk. The SCD cDNAs of different species, including hamster, rat, mice, human and sheep, have been cloned, but only one SCD gene isoform has been identified in sheep, located on chromosome 22 (Ward *et al.* 1997).

A remarkable variability in CLA content has been detected in a backcross Lacaune × Sarda population: individual variance accounted for 29.7% of the total phenotypic variance for CLA milk content and 34.4% for the CLA/vaccenic acid ratio, while sire variances were smaller (8.4% and 5.6%, respectively, for CLA milk content and the CLA/vaccenic acid ratio). Moreover, a QTL affecting the CLA/vaccenic acid ratio was found on OAR 22 (i.e. on the chromosome where the SCD gene is located; Carta *et al.* 2003). This result suggests a possible relationship between SCD gene polymorphism and its expression level, with a consequent effect on the quantity of CLA produced in the mammary gland from vaccenic acid. These findings open a very interesting scenario for the future implementation of molecular genetics in the selection of milk quality traits.

Table 13.2 Quantitative trait loci (QTLs) reported for dairy sheep

Trait	Location	Breed	Source
CLA/vaccenic acid in milk fat	OAR22	Sarda × Lacaune BC	Carta <i>et al.</i> (2003)
Milk fat content	OAR20	Sarda × Lacaune BC	Carta <i>et al.</i> (2003)
Milk protein content	OAR6	Churra	Diez-Tascon <i>et al.</i> (2001)
Milk protein content	OAR1	Sarda × Lacaune BC	Carta <i>et al.</i> (2003)
Milk yield	OAR3	Sarda × Lacaune BC	Carta <i>et al.</i> (2003)
Resistance to nematodes	OAR2, OAR6	Sarda × Lacaune BC	Moreno <i>et al.</i> (2006)
Somatic cell score	OAR6	Sarda × Lacaune BC, Lacaune	Rupp <i>et al.</i> (2003)
Teat placement	OAR9, OAR14	Sarda × Lacaune BC	Casu <i>et al.</i> (2003)
Udder attachment	OAR12	Sarda × Lacaune BC	Casu <i>et al.</i> (2003)
Fat yield	OAR7	Sarda × Lacaune BC	Casu <i>et al.</i> (2009)
Protein yield	OAR7	Sarda × Lacaune BC	Casu <i>et al.</i> (2009)

Source: Macciotta *et al.* (2005).

Somatic cell count (SCC) is an important trait indicating the susceptibility of animals to mastitis and it may indirectly improve the quality of the milk. However, indirect selection against mastitis by using SCC as an indicator trait is constrained by the low values of heritability and by its large standard errors, also demonstrated in dairy cattle (Macciotta *et al.* 2005). At present, only the Lacaune breeding programme takes SCC into account. An alternative approach could be the inclusion of SCC in an aggregate genotype that considers other functional traits related to milk production such as udder morphology, speed of milking and persistency of lactation. In particular, udder conformation is a trait that greatly affects the extent of manual intervention needed for extracting the milk retained in the udder (Casu *et al.* 2003). A linear scale for the morphological appraisal of the udder is currently used in Spain and Italy (de la Fuente *et al.* 1996, Casu *et al.* 2002). Genetic parameter estimates highlight a low to moderate genetic variability for the trait (Macciotta *et al.* 2005). Higher values found for the Sarda breed could be due to differences in the model used, particularly in the use of random contemporary groups, and in the size of the population analysed. Other measures, such as the size of the mammary cistern evaluated by ultrasound technique, could also be considered for genetic selection (Nudda *et al.* 2000).

Genetic engineering

Transgenic sheep for the growth hormone (GH) gene developed in Australia by the CSIRO produced twice as much milk than non-transgenic controls, but the milk had a lower concentration of protein, including casein (Bencini 2005). As the GH transgenic sheep produced the extra GH naturally, it should have been feasible to develop commercial applications. The transgenic sheep were destroyed due to animal welfare problems and changes in the legislation. Given the current attitude of consumers towards genetically modified organisms, it is unlikely that transgenic technology will be used to improve sheep milk production.

13.6.2 A non-genetic approach

The fat and protein content of milk from ewes with similar production levels can be improved, or worsened, by means other than genetics.

The variability is higher for fat vs. protein yield, with the total variance accounted for by the allometric model used to fit the data being 82–92% for fat and 92–98% for protein in the Sarda breed (Pulina *et al.* 2006a). Because the relationship between milk fat concentration and yield has a higher variability than that between milk protein concentration and yield (Fig. 13.1), modification of milk composition by non-genetic means should be easier to achieve for fat than for protein.

13.6.3 Nutrition

Among the most important nutritional factors affecting milk fat concentration and yield are the energy balance (EB) of the ewes, the concentration, intake and

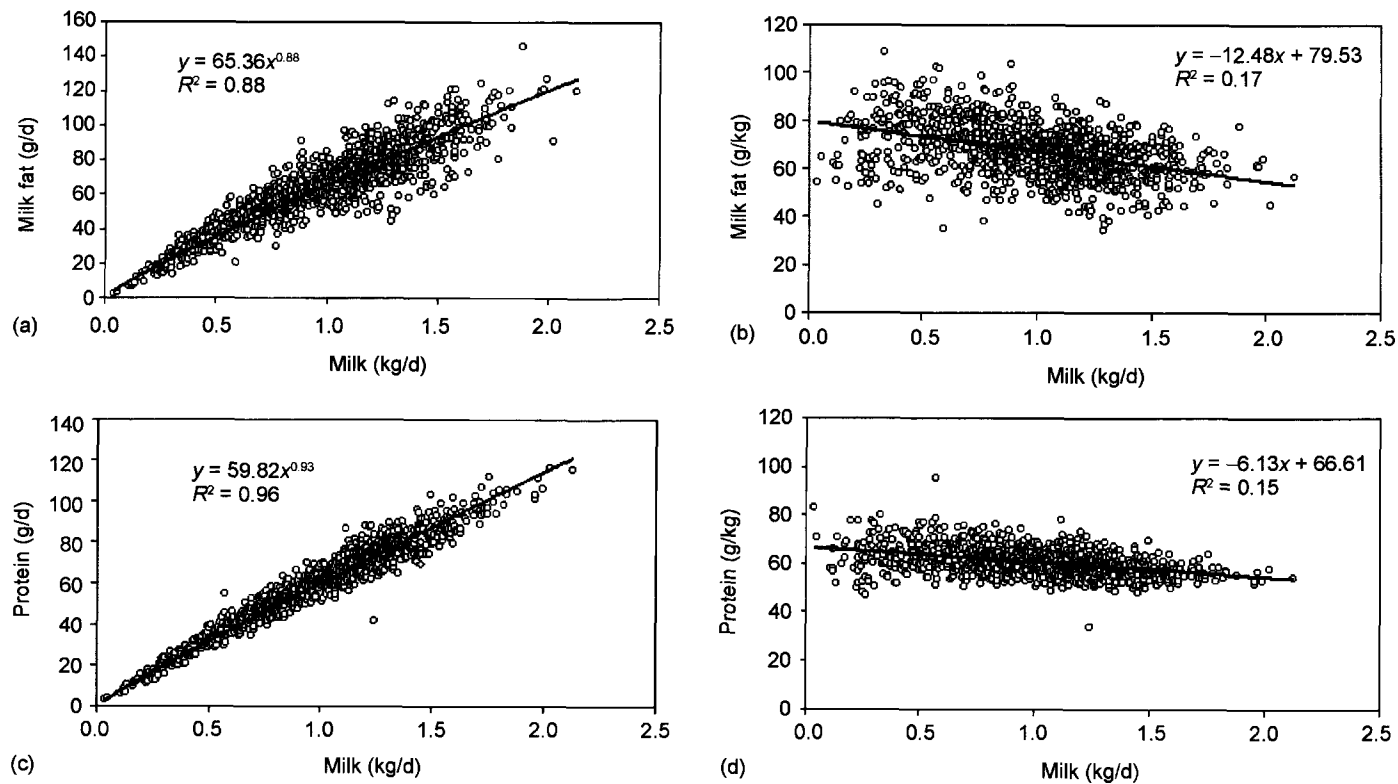


Fig. 13.1 Relationships between milk yield and (a) milk fat yield, (b) milk fat concentration, (c) milk protein yield and (d) milk protein concentration, based on 1665 individual data of Sarda ewes (adapted from Pulina *et al.*, 2006a).

source of dietary neutral detergent fibre (NDF) and non-fibrous carbohydrates (NFC), the particle size of feeds and fibre, as well as the amount, physical characteristics and FA composition of dietary fat supplements. Interactions among these factors and other less important ones make prediction of milk fat concentration complex.

Energy balance and milk fat and protein content

The energy balance (EB) of the sheep is probably the most important factor that affects milk fat concentration, especially in the early stages of lactation. A negative association between EB and milk fat concentration was reported for dairy cows (Palmquist *et al.* 1993) and sheep (Table 13.3). The similarity between the slopes of the two equations in Table 13.3 suggests that the rate at which mobilized body fat contributes to milk fat concentration is similar among breeds. However, the different intercepts suggest differences in basal fat concentration.

When the sheep mobilize body reserves, usually at the beginning of lactation, there is an increase in blood concentrations of long-chain FA (LCFA), derived from the mobilization of body fat triglycerides resulting in an increase of their concentration in the milk fat (Rossi and Pulina 1991). As ewes move from negative to positive EB, generally as lactation progresses, the milk fat LCFA fraction decreases.

The correlation between milk fat concentration and EB is weaker for ewes that have low milk yields, possibly because low-producing sheep are almost always in positive EB (Cannas and Avondo, 2002). Thus, milk fat concentration could be used to predict the EB of sheep or differences in EB in potentially problematic animals, which lose body condition too quickly (de Vries and Veerkamp 2000).

Milk protein concentration in sheep is positively associated with the energy concentration of the diet ($r = 0.64$; Bocquier and Caja 2001). Indeed, ewes with high milk yield fed high-grain and high net energy (NE) diets in early lactation had higher milk protein concentration and yield than ewes fed low-grain and low-energy diets (Susin *et al.* 1995). By contrast, no effects were reported on

Table 13.3 Relationship between daily energy balance (EB, Mcal of NEL_{1M} /day) and milk fat content (MF, g/100 ml) in sheep

Breed	Milk yield (l/day per head)	Fat (g/100 ml)	Equation	R^2	Source
Dairy and non-dairy breeds	0.650–3.500	4.2–9.8	$MF = 9.640 - 0.718 \times EB$	0.76	Bocquier and Caja (1993, 2001)
Comisana	0.650–1.600	4.0–10.6	$MF = 6.990 - 0.712 \times EB$	0.14 ($P < 0.0001$)	Cannas and Avondo (2002)

milk protein concentration in ewes fed pelleted diets, at two NE concentrations, in mid-lactation (Cannas *et al.* 1998).

Dietary fibre

The well-known association between the content of neutral detergent fibre (NDF) of the diet and milk fat concentration in dairy cows (Emery 1988) is also present in sheep (Table 13.4) and is also supported by the study of Nudda *et al.* (2004a), who reported a positive correlation ($r = +0.38$) between dietary NDF concentration and milk fat concentration. However, different results were observed in sheep grazed outdoors or fed indoors, possibly due to the different feeding regimes. In sheep fed indoors, an increase in NDF concentration caused a reduction of DM intake (DMI), net energy (NE) intake and milk yield, possibly because sheep fed these diets can select very little among the dietary ingredients (Serra 1998). By contrast, in grazing sheep, ewes with higher milk yield eat more pasture (and fibre) to meet their nutrient requirements. As intake of NE increased, due to the increase in pasture and NDF intake, the EB improved, causing a decrease, or interruption, of body fat mobilization. This probably reduced the availability of LCFA to the mammary gland, contributing to a decrease in the synthesis of milk fat (Cannas and Avondo 2002).

Fat supplements

Fat supplements can be added to the diet of sheep to increase dietary energy concentration, or to modify the concentration of milk fat, or its FA profile. Fat supplements can be protected or unprotected from rumen microbial activity.

High concentrations of fat added to diets can reduce the rumen fermentation activity of the bacteria and protozoa, thus reducing the advantage of the higher energy density of the diet (Palmquist and Jenkins 1980). Alteration of rumen microbial activity might also reduce milk fat synthesis, reduce the synthesis of

Table 13.4 Relationship between the dietary content of Neutral Detergent Fibre (NDF, % of DM) and milk fat content (MF, g/100 g) in sheep

Breed	Milk yield (kg/day per head)	Feeding	Equation	R^2	Source
Sarda		Indoors	$MF = 4.59 + 0.05 \times NDF$	0.23	Pulina and Rassu (1991)
7 dairy breeds	> 1.5 ^a	Indoors	$MF = 1.89 + 0.012 \times NDF$	0.48	Serra (1998)
Comisana	> 1.5 ^a	Grazing	$MF = 15.157 - 0.022 \times NDF$	0.36	Cannas and Avondo (2002)
Dairy breeds	> 1 ^a	Mostly indoors	$MF = 1.68 + 0.014 \times NDF$	0.43	Pulina <i>et al.</i> (2006b)

^a Below this production level there was no association between NDF and milk fat.

short-chain FA in the udder, and decrease the availability of metabolites (mainly FA from lipoproteins) that the mammary gland takes up from the blood.

Rumen-protected/inert fat supplements

The most commonly used rumen-protected/inert fats are calcium soaps of fatty acids (CSFA), and the most used CSFA is the calcium soap of palm oil (CSPO). A review of experiments in which CSPO was used in lactating sheep demonstrated that addition of CSPO to the diet caused consistent increases in milk fat concentration and yield (Pulina *et al.* 2006a). In most cases, CSPO caused reductions in milk protein concentration and yield, and sometimes the reduction was quite strong (for example, -17%; Horton *et al.* 1992). However, protein yield was not always reduced. CSPO interacts with the non-structural carbohydrate (NSC) source to influence milk fat content and yield. The stage of lactation and duration of the experiments influenced strongly the variability of the results among the different experiments. The variability in the results among experiments may also be due to differences in feeding techniques (e.g. grazing vs. indoor feeding), in the amount of pasture eaten in grazing studies, and in the way the supplement was fed (e.g. mixed in the concentrate supplied at milking or added to a total mixed ration).

Inclusion of other inert rumen fat sources, such as the calcium soap of olive oil, induced effects on milk fat similar to those reported for the calcium soap of palm oil. In one study by Kitessa *et al.* (2003) supplementation of inert polyunsaturated FA from tuna oil did not affect milk yield, or its fat, protein and lactose composition, over a 10-day feeding period in different sheep breeds. Although the inclusion of dietary fat has been reported to induce a reduction in milk protein synthesis, very little information is available on its effects on milk protein composition in sheep (Goulas *et al.* 2003, Zhang *et al.* 2006).

Rumen-unprotected fat supplements

The addition of unprotected lipids to the diet of dairy ewes has produced contrasting results on the content of their milk fat (Table 13.5). For example, Zervas *et al.* (1998) observed that the addition of 50 g/kg soybean oil to the concentrate increased milk yield and decreased milk fat and protein concentrations. Similarly, Sarda ewes supplemented with soybean oil (38.5 g/kg of DM) at two forage to concentrate ratios (75:25 and 60:40), had a significant increase of milk and fat yield, a similar fat concentration, and a lower protein concentration compared to the non-supplemented control ewes (Mele *et al.* 2002). In Merino and Awassi ewes in mid-lactation, supplementation with complete pelleted diets containing 25 and 50 g/kg of sunflower oil had no effects on milk yield, or milk fat and protein content, and the clotting properties of the milk did not differ at any level of sunflower oil (Bencini 2005). Addition of ensiled crude olive cake (150 g/kg of DM for a mean addition of 12 g/kg of oil to the diet) did not influence milk yield, but increased milk fat content by 12% (Hadjipanayiotou 1999). The source of fat supplemented (e.g. flax and sunflower), and its physical form (seed or oil), may be a reason for differences in

Table 13.5 Effects of free oil supplements on milk yield, milk fat content, fat yield, milk protein content and protein yield in dairy sheep. Differences are expressed in percentages with respect to control

Lipid source	Dose (g/d)	Milk yield	Fat content	Fat yield	Protein content	Protein yield	Reference
Olive oil	50	+29.2	-15.6	+8.7	-7.6	+19.6	Zervas <i>et al.</i> (1998)
Olive oil	42	+4.9	+12.0	+15.8	nd	nd	Hadjipanayiotou (1999)
Olive oil	100	-4.8	-22.9	-25.7	-14.0	-21.7	Porcu <i>et al.</i> (2003) ^a
Olive oil	148	+13.6	-2.2	+17.2	-3.8	+8.5	Gómez-Cortés <i>et al.</i> (2008)
Soybean oil	100	+4.4	+0.3	+13.3	-3.9	+8.2	Mele <i>et al.</i> (2002)
Sunflower oilseeds	78	-3.8	-3.9	-7.6	-2.3	-6.3	Zhang <i>et al.</i> (2006)
Flax oilseeds	68	+8.4	-2.2	+6.1	-1.1	+7.2	Zhang <i>et al.</i> (2006)
Tallow 0-21 DIM	175	+15.7	-14.4	-1.8	-12.0	+1.9	Goulas <i>et al.</i> (2003)
Tallow 0-21 DIM	137	+6.4	-13.1	-8.8	+3.4	+10.1	Goulas <i>et al.</i> (2003)
Tallow + meth. 0-21 DIM	188 + 13	+37.3	-5.4	+26.0	-10.1	+23.4	Goulas <i>et al.</i> (2003)
Tallow + meth. 22-91 DIM	155 + 11	+34.4	-12.4	+10.6	+0.8	+35.3	Goulas <i>et al.</i> (2003)

^a The values for Porcu *et al.* (2003) refer to the last two experimental days.

responses among different studies. The differences between the fat sources are likely to be related to their FA composition and/or their different seed size: sunflower seeds are much larger than flax seed, and this might have influenced rumination and rumen passage, thus affecting the availability of oil in the rumen (Zhang *et al.* 2006). Supplementation of lactating ewes with animal fats decreased milk fat concentration but did not change fat and protein yields (Goulas *et al.* 2003). When the same fat source was used with a source of rumen-protected methionine, there was a marked increase in milk yield, milk fat and milk protein yield in early lactation, but these effects were less pronounced in mid-lactation (Goulas *et al.* 2003).

Milk fat depression syndrome in sheep

A drastic reduction in the concentration of milk fat, also known as milk fat depression (MFD), occurs when dietary fibre is low and unprotected plant oils or fish oils are fed. MFD in dairy cows was positively associated with *trans*-10, *cis*-12 CLA concentration in milk fat (Bauman and Griinari 2003). Compared to dairy cows, MFD in dairy sheep is less common. No reduction in milk fat concentration was observed upon changing NDF level (240 vs 440 g/kg of DM; Nudda *et al.* 2004b) or the forage:concentrate ratio (60:40 vs. 75:25 supplemented, or not, with soybean oil; Mele *et al.* 2002); indeed, fat addition increased milk fat yield. Even the use of pelleted diets with very low fibre as the only feed did not induce MFD (Rossi *et al.* 1991). A reason why MFD is not common in ewes may be that they are able to ruminate and have normal rumen function when fed finely ground diets that cause rumen acidosis in cows (Cannas 2004). By contrast, in dairy sheep fed low fibre diets, supplementation with 100 g of free olive oil for one week did not modify milk yield, but it decreased milk fat and protein concentration and yield in the last two experimental days (Porcu *et al.* 2003). These different results may be due to an interaction between low fibre and high oil addition, which are the two conditions necessary to induce MFD (Bauman and Griinari 2003).

Dietary protein

Attempts to increase milk protein content through dietary crude protein (CP) supplementation have often resulted in no effects, or in small changes (Bocquier and Caja 2001). Diets with an increasing dietary CP concentration, at two NE levels, did not change the milk protein concentration but did increase milk protein yield (Cannas *et al.* 1998). However, protein concentration and daily protein yield increased in the milk of ewes in which the dietary CP concentration was initially very low (i.e., 100–110 g/kg DM) and was increased up to 140 g/kg DM (Calderon Cortes *et al.* 1977, Cowan *et al.* 1981). Increases in dietary protein generally are accompanied by increases in food intake. The consequent increase in milk production masks the increased synthesis of protein in the mammary gland, and the concentration of protein in the milk does not change (Robinson *et al.* 1974, Cowan *et al.* 1981, Penning *et al.* 1988, Frey *et al.* 1991, Hatfield *et al.* 1995).

Dietary protein sources can influence the yield and protein concentration of sheep milk as a result of the balance between the dietary essential amino acids and their requirement by dairy ewes. Fish meals have an amino acid composition that is very close to optimal for milk protein synthesis compared to other protein sources, such as soybean meal, linseed or peanut proteins. This explains the highest milk, and milk protein, yield in ewes fed fish meal (Purroy and Jaime 1995, Gonzales *et al.* 1982).

Pulina *et al.* (2006a) reviewed the effects of supplementation of methionine (Met) and lysine (Lys) on milk yield and protein content in ewes. In sheep, supplementation of ruminally protected Met and Lys was used to increase the efficiency of N utilization for milk protein synthesis (Lynch *et al.* 1991), and supplementation caused a slight increase of the Met and Lys content in milk, which was associated with a higher growth of lambs suckling this milk.

The protein content of the diet affects the quantity and the partition of nitrogenous substances in the milk. In general, an increase of dietary crude protein (CP) did not affect milk CP content, but influenced the milk N fraction. In sheep, there is a close positive relationship between milk urea (MU) and dietary CP concentration (Fig. 13.2, Cannas *et al.* 1998), probably because the efficiency of utilization of dietary N for milk protein synthesis decreased, and N losses increased, as CP content in the diets increased.

In addition, high-energy diets reduce the necessity for ewes to use amino acids as an energy source, so that less ammonia is produced from amino acid catabolism. Effects of dietary rumen-degradable organic matter and rumen-degradable CP on milk urea (MU) were examined by Landau *et al.* (2005), who found differences of about 5–6 mg/dl of MU, depending on the ratio of rumen-degradable CP to rumen-degradable organic matter, for similar dietary CP concentrations.

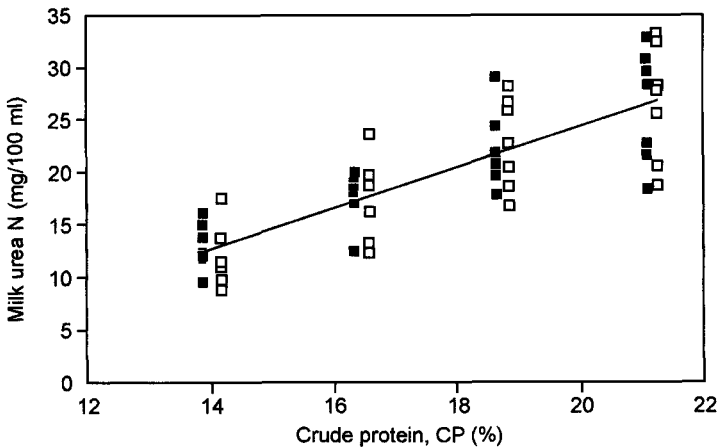


Fig. 13.2 Relationship between the concentration of milk urea N and the percentage of dietary crude protein in ewes fed diets with high energy (□) and low energy (■) total mixed ratios (adapted from Cannas *et al.*, 1998).

Alternative feed sources

The use of alternative feeds (AF) in sheep nutrition has been reviewed by Vasta *et al.* (2008). Several researchers have shown that AF can be used successfully as supplements for sheep nutrition as a replacement for concentrates, without compromising animal performance. Some shrubs, legume seeds (e.g. *Pisum sativum*, *Cicer arietinum*, *Vicia faba*, *Ceratonia siliqua*), local agro-industrial by-products (e.g. olive cake, sugar beet pulp, extruded linseed cake and citrus pulp) or novel pasture species are widely available in Mediterranean countries and can reduce nutrition costs. Many of these alternative feeds contain secondary compounds, such as tannins. The effect of condensed tannins (CT) on milk composition depends on the CT concentration in the diet. When present in high concentrations in the diet, tannins can negatively affect animal productivity because they markedly reduce rumen microbial activity and bacterial and feed amino acid digestion in the intestine. In low amounts, tannins tend to increase milk yield and protein content, probably because they protect dietary proteins from ruminal degradation and increase the availability of essential amino acids. Moreover, the inclusion of alternative feeds in the diet can influence the appearance of nutraceutical or flavour-active compounds in milk and in dairy products. CT from *Hedysarum* (40 g/kg DM) offered to lactating sheep decreased the intestinal absorption of indole and skatole (Roy *et al.* 2004a) and their secretion in milk (Roy *et al.* 2004b). An increase in the concentration of polyunsaturated fatty acids (PUFA) in sheep milk was reported in dairy ewes grazing on *Hedysarum coronarium* compared to sheep fed on annual ryegrass, probably because the tannins in the *Hedysarum* reduced the biohydrogenation of PUFA in the rumen (Addis *et al.* 2005).

Vasta *et al.* (2008) also reviewed the use of alternative protein sources for dairy sheep. The partial or total replacement of soybean meal with chickpeas in the diets of sheep in mid-lactation did not affect milk yield and composition (Christodoulou *et al.* 2005). When soybean meal was substituted by isoproteic amounts of *Lupinus albus* in highly productive Sarda ewes in mid-lactation, no differences in milk yield, milk fat and protein content were observed between the two protein sources (Masucci *et al.* 2006). The use of agro-industrial by-products for sheep nutrition is an attractive proposition for sheep dairy farmers. Olive cake containing 115 g of fat/kg DM was used as silage to replace grass hay in the diet of Sarda ewes and did not modify milk yield or composition (Cabiddu *et al.* 2004).

The replacement of a starch-rich concentrate (500 g/d) with soybean hulls (350 g/d) and brewers grains (150 g/d) in the diet of dairy sheep increased milk yield and milk fat concentration but tended to decrease milk protein concentration (Cavani *et al.* 1990). Similarly, milk yield and milk fat concentration increased and milk protein concentration decreased when soybean hulls and beet pulps replaced corn and wheat grains in ewes in mid-lactation (Cannas *et al.* 1998). Zenou and Miron (2005) reported similar results on the effect of soybean hulls on milk yield and milk fat concentration in Assaf ewes. The replacement of grains, soybean meal, and wheat middlings with dried citrus pulps (300 g/kg of

concentrate DM) in the diet of low-productive dairy ewes did not affect milk yield or milk fat and protein concentrations but modified milk FA profile (Fegeros *et al.* 1995). The inclusion of increasing doses of citrus fruit (up to 30% on DM basis) in substitution of barley and beet pulp did not affect the milk composition or the biochemical and sensory characteristics of cheese (Jaramillo *et al.* 2009). By contrast, the partial replacement of an oat hay and concentrate mixture with silage made from oranges (400 g/kg of dietary DM) in the diet of dairy ewes decreased milk yield and milk protein concentration, probably due to a reduced microbial protein synthesis and flow to the intestine (Volanis *et al.* 2004). The supplementation of wet lemon (*Citrus limon* L.) pulp to Valle del Belice ewes grazing on natural pastures positively influenced milk yield and decreased milk protein content but did not modify milk fat concentration and milk coagulation properties (Todaro *et al.* 2004).

The use of novel forage and pasture species to increase pasture quality and availability in late spring and early summer has not been fully investigated in the Mediterranean areas. Grazing Sarda ewes on safflower, chicory and burr medic produced no differences in milk yield among the three species, while safflower and chicory-fed ewes produced milk with lower fat and protein concentrations than ewes fed on burr medic. The safflower diet caused a higher reduction of milk fat and protein concentration than the chicory diet, probably because of its higher content of total polyphenols and tannins compared to that of the other two species, which might have reduced rumen microbial activity (Landau *et al.* 2005). The above results highlight the fact that the use of alternative sources of protein is quite site specific and results can vary depending on many different factors, but especially on the quality of locally available food sources.

Pasture

Forage quality and its availability have significant effects on milk composition. The type of forage, its conservation and botanical composition all influence milk quality.

Pulina *et al.* (2005) re-elaborated the results of 43 groups of lactating Sarda sheep from 18 different experiments in animals fed exclusively on pasture (P), compared to grazing ewes supplemented by 0.5 kg/d of concentrate (P+C) and ewes fed exclusively a complete pelleted diet (C). Supplementing grazing with concentrates did not increase milk production when compared to grazing alone. Higher milk yield generally resulted in lower fat and protein concentrations, so that the differences in daily yield of total utilizable substances (TUS = the quantity of fat and protein produced daily by a single animal) were lower (Table 13.6). However, ewes fed a complete pelleted diet produced more TUS than grazing sheep ($P < 0.001$) because they had significantly higher intakes. This resulted in a favourable efficiency feeding index, but the feeding cost per kilogram of total utilizable substances (TUS) produced was much higher than in regimes which used only grazing. This result justifies the growing importance of pasture as a food source, given that intensive systems have lower labour costs

Table 13.6 Food intake, milk yield and composition, efficiency feeding index and feeding costs of Sarda dairy ewes under three different feeding regimes

Feeding	Number of trials, animals	Intake (g/d)	Milk yield (g/d)	Milk fat (%)	Milk protein (%)	Efficiency feeding index (DM/milk) (kg)	Cost of TUS ^a (euro/kg)
Complete pelleted diets	12, 86	2497 ^b	1791 ^b	5.57 ^c	5.38 ^c	1.419	3.83 ^b
Pasture + concentrates	12, 744	1850 ^c	1251 ^c	6.48 ^b	5.84 ^c	1.677	2.04 ^c
Pasture	19, 1078	1704 ^c	1087 ^c	6.56 ^b	5.88 ^b	1.712	1.35 ^d
<i>P</i> value		<0.001	<0.001	0.003	0.057	n.s.	<0.001

^a TUS = total utilizable substances = fat + protein yield in kg. Cost (euro/kg) of DM: pasture 0.1, concentrates 0.3.

^b, ^c and ^d indicate significant differences at the levels shown in the last line.

but higher feeding and structural expenses per head. A mixed feeding regimen seems to be a good compromise, thanks to the interesting economic conversion index and the productive level achievable in groups fed on grazing supplemented with concentrates. The concentrate/pasture substitution index, that is the reduction in the amount of grass DM voluntarily eaten by a sheep supplemented with 1 kg of concentrate DM, is on average 0.69. This explains the positive, even if not significant, effect on milk yield of using concentrate supplements rather than grazing alone.

Pasture can also affect the flavour, antioxidant properties and fatty acid profile of the milk and dairy products derived from it. For instance, the botanical composition of pastures influences the proportions and quantities of monoterpenes and sesquiterpenes in milk fat and, consequently, plays a role in determining cheese flavour (Viallon *et al.* 2000).

Addis *et al.* (2006) observed that the inclusion of *Chrysanthemum coronarium* in a grass–legume based pasture composed of *Lolium rigidum* and *Medicago polymorpha* markedly influenced the occurrence of terpenes (terpinolene, tricyclene and 3,7-dimethyl-1,6-octadiene) in the volatile fraction of sheep milk and cheese, and that cheese obtained from the milk of sheep grazing a mixture of ryegrass, *Medicago polymorpha* and *Chrysanthemum coronarium* could be distinguished from that made with the milk of sheep grazing a mixture of ryegrass and *Medicago polymorpha*. Similarly Carbonell *et al.* (2002) found the highest concentrations of terpenes, other aromatic compounds (e.g. toluene, naphthalene and phenol) and free FA in the cheese made from sheep milk produced in spring.

Sheep on pasture produce a milk with a higher content in liposoluble vitamins (A, E and D), which are then efficiently transferred into the cheese made from the milk (Lucas *et al.* 2006). Fresh forages also influence the fatty acid profile of milk fat. In general, the milk from pasture-fed ewes has a higher content in PUFA because grass lipids contain a high proportion of PUFAs compared to dry diets (Clapham *et al.* 2005).

In dairy ewes that are grazing pasture, the concentrations of C18:2, C18:3, *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk were higher in April than in May (Nudda *et al.* 2003), possibly due to the decrease in forage quality that normally occurs as Mediterranean pasture plants mature from the vegetative to the reproductive stage. In cheese-making factories, bulk milk, cheese and ricotta analysed from March to June also showed a decrease in *cis*-9, *trans*-11 CLA and in *trans*-11 C18:1 content with season (Fig. 13.3, Nudda *et al.* 2005).

Levels of PUFA and CLA in milk are also affected by the botanical composition of the pasture. Higher levels of milk C18:3, *trans*-11 C18:1 and CLA were obtained when diets were based on pure legumes or grass–legume mixtures compared with pure grass pastures (Cabiddu *et al.* 2005). In a study comparing four forage species, the content of *cis*-9, *trans*-11 CLA in milk of ewes consuming *Chrysanthemum coronarium* and *Medicago polymorpha* was higher than in milk from sheep fed *Lolium rigidum* and *Hedysarum coronarium* (Addis *et al.* 2005).

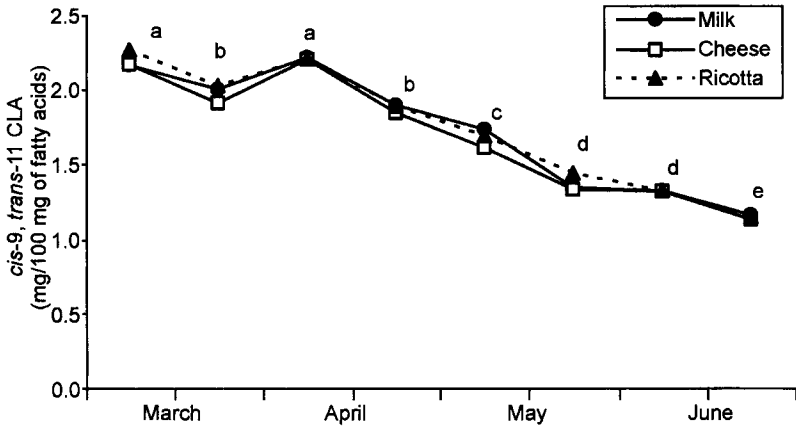


Fig. 13.3 Seasonal evolution of *cis*-9, *trans*-11 CLA in sheep milk, cheese and ricotta sampled every two weeks from March to June in two milk processing plants in North Sardinia (adapted from Nudda *et al.*, 2005). a–e indicate significant ($P < 0.05$) differences between data points.

Designer milks with increased concentrations of C18:3 *n*-3 and CLA can be produced by supplementing the sheep diet with linseed oil, soybean oil or sunflower oil (Nudda *et al.* 2006, Mele *et al.* 2006, 2007, Bencini 2005).

13.6.4 Nutritional imbalances and SCC

The SCC in milk is indirectly affected by the nutrition of the ewe. For example, poor nutrition may predispose ewes to metabolic and infectious health disorders, which increase the susceptibility of the mammary gland to inflammation. Correct integration of vitamin A, or beta carotene, and vitamin E and Se in the ration maintains immune responses of mammary gland cells, thereby reducing the incidence of infections that can lead to increases in milk SCC (Morgante *et al.* 1999). Integration of the diet with these substances is particularly useful when ewes are fed mainly conserved fodders (i.e., hay or silage) that may have undergone considerable losses in beta-carotene and vitamin E during storage. Selenium deficiency can also compromise the antioxidant properties of the mammary tissue and cause an increase in the somatic cell count of the milk (Ronchi *et al.* 1994).

Improvement of the quality of the diet can reduce the somatic cell count in the milk, especially toward the end of lactation (Pulina *et al.* 1992). This is probably due to an improved functionality of the rumen, which results in the combined effect of increasing production, resulting in a dilution of the somatic cells and a slowing down of mammary cell turnover. Somatic cell counts can increase in sheep that are grazing green pasture due to excessive nitrogen intake (Cuccuru *et al.* 1994). The use of regulators of rumen fermentations such as Flavomycin (Hoechst Ltd) may result in reductions in the somatic cell count (Pulina and Rassa 1991).