Lipid requirements in the nutrition of dairy ewes

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ABSTRACT

The aim of this review was to contribute to the knowledge of lipid requirements in dairy ewes, by reviewing experimental papers about lipid supplementation in dairy ewe feeding. The number of trials in ewe feeding is lower than that in dairy cow feeding and, leaving calcium soap of palm oil out of consideration, there is a lack of knowledge regarding the effects of protected and unprotected lipid sources on milk yield and quality from dairy ewes. On the basis of data reported in the literature, the optimal dose of calcium soap of palm oil resulted to be 100-120 g/d. Also, milk fatty acid composition may be improved by adding calcium soap of fatty acids to ewe diets. The general effect of calcium salt supplementation is an increase in milk unsaturated fatty acids and a decrease in saturated ones. However, more research is needed in order to explain the effect of different fat sources (protected and unprotected) on milk yield and quality from dairy ewes.

Key words: Dietary fat, Lactating ewes, Milk quality, Fatty acids.

RIASSUNTO

FABBISOGNO DI LIPIDI NELL'ALIMENTAZIONE DELLA PECORA IN LATTAZIONE.

Lo scopo di questa rassegna è quello di fornire un contributo alla conoscenza del fabbisogno alimentare di lipidi delle pecore in lattazione mediante l'analisi dei risultati delle ricerche eseguite sull'argomento, dalle quali sono state tratte le informazioni sulla composizione della dieta e sulla produzione quanti-qualitativa di latte. Dai lavori sperimentali pubblicati sull'argomento emerge chiaramente che, fatta eccezione per i saponi di calcio dell'olio di palma, per i quali sono disponibili un numero sufficiente di dati, mancano ancora molte informazioni relative all'utilizzo delle diverse fonti lipidiche protette e non e sul loro effetto sui parametri quantitativi e qualitativi del latte di pecora. Ad ogni modo, da quanto reperibile in letteratura, è possibile stabilire tra i 100 e i 120g/d la dose di saponi di calcio dell'olio di palma in grado di influenzare positivamente la produzione di latte e il suo contenuto in grasso. Anche la composizione in acidi grassi del latte di pecora può essere modificata attraverso l'utilizzo dei saponi di calcio degli acidi grassi, che, come effetto generale, comportano un aumento degli acidi grassi insaturi e una diminuzione di quelli saturi a media catena.

Parole chiave: *Grasso nella dieta, Pecore in lattazione, Qualità del latte, Acidi grassi.*

Introduction

Fats can be added to dairy cow and sheep diets with the aim to meet the high energy require-ments of the animals during the first phase of lactation, to increase milk fat content and to modify the milk fatty acid profile (Palmquist and Jenkins, 1980).

The interest to include fat in diets for ruminants is not recent. In 1907, at the dawn of modern animal science, Kellner, studying the data from ten European experimental centres, showed that no beneficial effects on milk production were present when the dairy cow diets were supplemented with fats (Palmquist and Jenkins, 1980). On the contrary, between 1920 and 1940 Maynard and Loosli observed an improvement in milk yield of about 2-10% for cows fed supplements with 4-7% of fats with respect to those fed rations with a lipid content of about 1-3% (Palmquist and Jenkins, 1980). In 1960, Warner showed that fat supplements in diets for ovines reduced fibre digestibility (Palmquist and Jenkins, 1980).

In 1988, Smith (Palmquist, 1994) summarized the role of fats in ruminant nutrition as follows: i) to increase gross energy intake when dry matter contribution is limited; ii) to increase net energy optimising the loss of heat; iii) to modify milk fat composition with the transferring of dietary fatty acids into milk; iv) to partially replace the highly fermentable carbohydrates. All depend on several factors such as: i) digestibility of fats added to the ration; ii) influence of this addition on voluntary intake and on digestibility or metabolic utilisation of other components in the ration; iii) kind of fat added.

Fats in ruminant nutrition

Conventional ruminant diets rarely contain more than 3.5% of crude fat out of which about 20-50%, influenced by grain or forage presence is not represented by fatty acids. In fact, in leaves the crude fat is waxes, pigments as chlorophyll and other unsaponifiable substances (Palmquist and Jenkins, 1980).

The amount of fat added to the diet may affect dry matter intake (DMI); in fact if dietary fat supplementation exceeds 6%, DMI can decrease (Palmquist, 1994). The physiological factors that may regulate this behaviour are not clear, but several hypotheses have been proposed: i) feeds with high content of fat enhance the production of cholecystokinin that inhibits DMI (Rehfeld, 1989); ii) the presence of a high content of fatty acids in the diet can favour the increase of b-oxidation in

the liver limiting, as a consequence, DMI (Scharrer and Langhans, 1986); iii) in the gut the level of enterostatin can increase and DMI can decrease (Erlanson-Albertsson, 1992); iv) the synthesis of apolipoprotein A-IV, which is responsible for the satiety signal, is enhanced (Emery *et al.*, 1992). The hypothesis ii) seems to be confirmed for animals in the first phase of lactation. During this period, in fact, the energy requirement increases and endogenous lipid reserves are mobilised as a consequence of a negative energy balance. This process leads to an increase in the plasma content of non-esterified fatty acids (NEFA) and of b-oxidation in the liver. If the percentage of fats in the diet is high, this catabolism path is enhanced, especially if the animals are too fat at the time of calving or lambing.

Lipids, moreover, are able to modify the ratio between volatile fatty acids (AGV) in the rumen (perhaps as a consequence of a negative effect on cellulolytic rumen bacteria rather than amylolytic microbe), which induces an increase in propionate (Doreau *et al.*, 1991). Another effect is the interference with diet digestibility. Devendra and Lewis (1974) summarized four theories to explain this effect: i) the bacterial approach on feed particle surface is obstructed by the physic assemblage of fibre with fat; ii) the toxic effect of lipids on microbial population in the rumen; iii) the inhibition of the normal rumen bacterial activity because of the negative effects of fatty acids on the surface of microbial membranes; iv) the decrease in cations concentration in rumen liquor, as a consequence of the formation of insoluble salts with long chain fatty acids, causing a variation of ionic activity and of rumen liquor pH, which affects bacteria population. Experimental trials on pure bacteria cultures showed that fatty acids are able to link bacteria and protozoa cells. A higher content of fibre in the diet can limit this phenomenon by interfering on the linkage between the microbial cell and fat (Czerkawski, 1973; Harfoot *et al.*, 1974; Henderson, 1973). Also the presence of metallic ions is able to improve digestibility. Brooks *et al.* (1954) showed that the ash of alfalfa hay limits the negative effect of maize oil on digestibility for the presence of calcium. Johnson and McClure (1973) demonstrated that the addition of limestone in ewe and calve diets

prevents the decrease in digestibility when animals are fed with fat and maize silage. El Hag and Miller (1972) discovered an increase in digestibility by adding $CaCl_2$ on cellulose and hemicellulose deriving from distillation residuals. A hypothesis to explain calcium effect is that insoluble salts between calcium ions and fatty acids are formed thereby diminishing the quote of fatty acids that interfere with bacteria (Palmquist, 1994). Similar behaviour is demonstrated by Mg and Ba. When dairy ruminants are fed diets supplemented with 5-6% of fat on DM, milk fat content often increases. However, if a higher percentage of fat (especially if polyunsaturated fatty acids are present) is included in a ration with a low content of fibre, milk fat can decrease because: i) unprotected fat can negatively influence rumen bacteria metabolism (Palmquist, 1994); ii) several long chain fatty acids are able to negatively affect the metabolism of mammary gland enzymes (Park *et al.*, 2000); iii) fats are able to inhibit the enzymes responsible for mammary gland uptake of fatty acids from plasma (Palmquist, 1994). To avoid negative effects on milk fat production, several methods of fat rumen protection have been used. A common technique to protect fat from rumen fermentation is the coating of fat (sprydried) with proteins and formaldehyde. For this kind of protection, better results are obtained from *in vitro* trials than from in vivo ones, because the effect of mastication could break feed particles and the film protecting the fat (Doreau *et al.*, 1991). Literature shows that high hay diets supplemented with 800 g/d of safflower oil protected with casein induced a milk fatty acid modification in dairy cows, leaving milk production parameters uninfluenced. However, several aromatic substances formed by oxidation processes are found in milk, but they can be removed by including α tocopherol (either free or as acetate) directly in the milk (Goering *et al.*, 1975).

Another technique of fat protection is the prilled fat that is made by fatty acids in a solid state. Since fat with a higher melting point is insoluble in the rumen liquor, an increasing amount of dietary fat escapes from rumen and arrives in the gut. Calcium soaps of fatty acids are also a good method for fat protection. In fact, in these molecules the carboxylic function is not free

and microbial enzymes are not able to link the molecule in order to start the biohydrogenation of double bounds. A free carboxylic function is an indispensable condition for microbial enzymes to block the molecular structure and start the reductions (Sukhija and Palmquist, 1990). *In vitro* experimental data showed a degradability of palm oil calcium soaps of about 47% (Chouinard *et al.*, 1998). Data of Sukhija and Palmquist (1990) showed that calcium soap hydrolysis is related to pH values (<6) and to the unsaturation grade of fatty acids present: PUFA calcium soaps are hydrolysed faster than SFA, but if buffers such as $NaHCO_3$ and $KHCO_3$ or MgO are added to the ration, triglyceride content in plasma and mammary gland uptake is increased (Thivierge *et al.*, 1998).

Fats in dairy ewe nutrition

In dairy ewes, calcium soaps of fatty acids are often used as a source of lipid supplementation (Perez-Hernandez *et al.*, 1986; Kovessy *et al.*, 1987; Pulina *et al.*, 1990; Rossi *et al.*, 1991; Casals *et al.*, 1992, 1999; Horton *et al.*, 1992; Chiofalo *et al.*, 1993; Laudadio *et al.*, 1997; Perez-Alba *et al.*, 1997; Todaro *et al.*, 1997; Espinoza *et al.*, 1998; Osuna *et al.*, 1998; Rotunno *et al.*, 1998; Sevi *et al.*, 1998; Antongiovanni *et al.*, 2002). Some experimental trials about the effects of unprotected fat as plant oil seeds or vegetable oil and animal fats on milk yield and quality have also been published (Laudadio *et al.*, 1997; Zervas *et al.*, 1998; Hadjipanayiotou, 1999; Kitessa *et al.*, 2001; Mele *et al.*, 2002). However, the number of trials in ewe feeding is more limited than that in dairy cow feeding.

Unprotected fats

Unprotected fat supplementation (such as vegetable oils or whole grains) in dairy ewe diets induces an increase in milk lipid content and total milk fat yield, that can exceed 2%, when ewes are fed oil seeds (Schmidely and Sauvant, 2001).

The increase in milk fat content seems to be higher in dairy ewes than in dairy cows and goats, as reported by Chilliard *et al.* (1993).

Although data reported in the literature are sometimes contrasting (Schmidely and Sauvant, 2001), it is, the general opinion that the inclusion of unprotected fat in dairy cow diets induces a decrease of milk fat content.

Although results in dairy sheep are more homogeneous, also in this species the inclusion of unprotected oil (as soybean oil) in the diets sometimes decreases milk fat content (-1.3%) or DMI (Zervas *et al.*, 1998).

However, the effects of fat supplementation are related not only to the amount of lipid included in the diet, but also to fat kind. Dairy ewes fed diets with a total fat content equal to 5.7% on DM by the inclusion of soybean oil induced a significant decrease in milk fat production (-1.3%) and of DMI (Zervas *et al.*, 1998); conversely the inclusion of a higher amount of fat, such as whole cotton seeds or sunflower seeds (total fat equal to 7% on DM), increases the milk fat content (Osuna *et al.*, 1998).

The results of Zervas *et al.* (1998), however, did not agree with those recently obtained by Mele *et al.* (2002), who fed dairy ewes a fat supplemented diet that contained an amount of unprotected soybean oil equal to 5.5% on DM. In this experimental trial the fat supplementation allowed an increase in both daily milk and fat yield as compared with the control diet (1.7 % on DM), while the milk fat concentration did not change.

Similar results were obtained feeding dairy ewes a diet with 3.3% of ether extract by including ensiled crude olive cake (Hadjipanayiotou, 1999).

The supplementation of unprotected fat in the diet of dairy sheep, therefore, did not seem to induce effects comparable to those reported for dairy cows. Recently, Bauman and Griinari (2001) proposed a new theory in order to explain the milk fat depression observed when dairy cows are fed diets supplemented with unprotected fat. This theory postulated that, under certain dietary conditions, the pathways of rumen biohydrogenation are altered to produce unique fatty acid intermediates, which are potent inhibitors of milk fat synthesis. On the basis of this theory, two conditions are necessary in order to induce the milk fat depression in dairy cows: altered rumen microbial processes and the presence of unsaturated fatty acids in the diet.

Actually, we cannot draw any conclusion about the possibility that the above mentioned theory may also be applied to dairy sheep because the data in the literature are not sufficient; therefore, more studies are needed in order to evaluate whether the interaction between dietary unsaturated fatty acids and the forage/concentrate ratio may negatively influence the milk fat synthesis in dairy sheep as well.

Protected fats

Several protection systems may be applied to dietary fat sources, but calcium salts of fatty acids are the most used in dairy sheep nutrition and represent the most common form of lipid supplementation.

Generally, the inclusion of calcium salts of palm oil in the diets of dairy sheep induces an average increase in milk fat content equal to 13% according to Schmidely and Sauvant (2001), who found a quadratic relationship between milk fat content and the amount of diet calcium soap of palm oil by reviewing 11 experimental trials:

$$G \% = 67.9 + 0.153SC - 0.00033SC^2,$$
$$r = 0.96; n = 42,$$

where G % = milk fat content; SC = amount of diet calcium soap of palm oil.

According to this relationship, the optimal level of calcium soap of palm oil should be 100-150 g/d, which allowed the highest increase of milk fat content (+30%); however, in the opinion of Caja and Bocquier (1998) the optimal dose should be lower (70-120 g/d).

The addition of calcium soap seemed to have little effect on milk yield (about +2%) and, when the dose exceeded 100 g/d, an unfavourable effect occurred (Schmidely and Sauvant, 2001).

A similar pattern for milk fat percentage and daily milk yield also resulted when Sarda and Comisana ewes were fed unipellets mixed with calcium soap of palm oil (Rossi *et al.*, 1991; Pulina *et al.*, 1995; Laudadio *et al.*, 1997) (Figures 1 and 2), although the data refer to a short range of calcium soap doses.

Figure 1.   Relationship between amount of diet calcium soaps of palm oil and milk fat percentage in Sarda and Comisana ewes (data from Rossi *et al.*, 1991; Pulina *et al.*, 1995; Laudadio *et al.*, 1997)

$$y = -0.0002x^2 + 0.068x + 2.5907$$
$$R^2 = 0.7526$$
$$P < 0.01$$

% milk fat

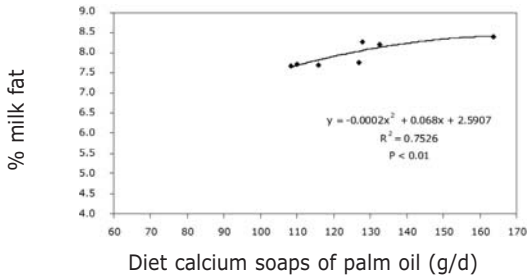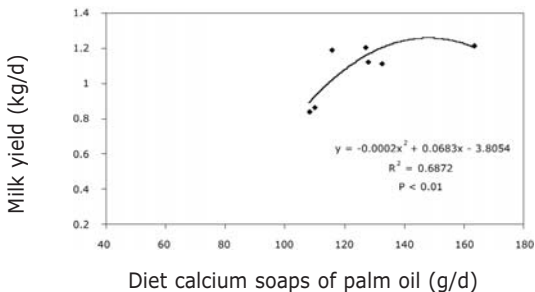Diet calcium soaps of palm oil (g/d)

Figure 2.   Relationship between amount of diet calcium soaps of palm oil and daily milk yield in Sarda and Comisana ewes (data from Rossi *et al.*, 1991; Pulina *et al.*, 1995; Laudadio *et al.*, 1997).

$$y = -0.0002x^2 + 0.0683x - 3.8054$$
$$R^2 = 0.6872$$
$$P < 0.01$$

Milk yield (kg/d)

Diet calcium soaps of palm oil (g/d)

Daily milk fat yield was also influenced by calcium soap of palm oil based on a quadratic relationship (Schmidely and Sauvant, 2001):

$$G = 130.5 + 0.285SC - 0.00084SC^2,$$
$$r = 0.98; n = 37,$$

where G = grams of milk fat per day; SC = amount of diet calcium soap of palm oil.

In this case the optimal dose of calcium soap also resulted 100-150 g/d, which allowed an increase in daily milk fat yield equal to 25 g/d.

Recently, Bocquier and Caja (2001) suggested that in order to optimise daily milk fat yield, the optimal doses of calcium soap of palm oil were 120 and 70 g/d, during lamb suckling and during lactation, respectively.

Several factors may influence the effects of lipid supplementation of dairy sheep, included the lactation phase. The inclusion of calcium soap during early lactation, in fact, made it possible to obtain better results (Perez-Alba *et al.*, 1997, Casals *et al.*, 1999) than lipid supplementation offered during the whole lactation phase (Rotunno *et al.*, 1998). This different answer to fat supplementation, observed in dairy sheep, might be related to the effects of the energy balance on the mammary gland that varies its utilisation efficiency of diet lipids. In particular, the energy balance status in the ewe can modulate the activity of both mammary and lipid tissue lipoprotein lipases and indirectly support the fatty acid transfer toward the mammary gland during early lactation, or toward lipid tissue in the second phase of lactation (Schmidely and Sauvant, 2001).

Lipid supplementation during late lactation may also cause a decrease in both milk protein content and total casein content (Bocquier and Caja, 2001).

All the effects described so far refer to calcium soap of palm oil, which represents the most common protected fat source in dairy sheep feeding, but in the Mediterranean area calcium soap of olive oil (a by product of the olive oil industry) may also represent a good fat source in ewe nutrition.

Although very few trials are available, the inclusion of 5-7% on DM of calcium soap of olive oil made it possible to obtain effects on milk fat similar to those reported for calcium soap of palm oil: daily milk fat yield and content resulted higher (from 5 to 8%), while milk yield resulted practically unchanged (Antongiovanni *et al.*, 2002).

Regarding milk protein content, results are discordant: Antongiovanni *et al.* (2002) reported a slight decrease (according to that which was reported for the calcium soap of palm oil supplementation), while other Authors did not observe this phenomenon (Perez-Alba *et al.*, 1997). When Sarda and Comisana ewes were fed unipellets supplemented with calcium soap of palm oil, a significant inverse quadratic relationship resulted (figure 3).

Figure 3. Relationship between amount of diet calcium soaps of palm oil and milk protein percentage in Sarda and Comisana ewes (data from Rossi *et al.*, 1991; Pulina *et al.*, 1995; Laudadio *et al.*, 1997).

$$y = 0.0007x^2 - 0.2177x + 20.98$$
$$R^2 = 0.8371$$
$$P < 0.01$$
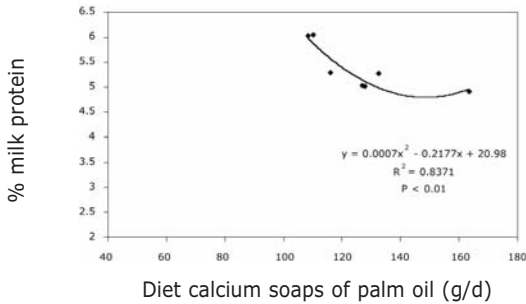
The decreased milk protein concentration with fat supplementation may be related to a combination of factors (Palmquist and Jenkins, 1980) such as: i) dilution effects, when milk yield was increased, ii) reduction of microbial protein synthesis, when DMI were depressed, iii) changes in glucose metabolism as suggested by Smith *et al.* (1978).

Lipid supplementation and milk fat quality

It is common knowledge that the nature of diet lipid sources may modify milk fatty acid composition. In particular, unsaturated fat sources may be able to increase the milk unsaturated fatty acid content, but if this kind of fat is not protected, it may imply an increase in the content of *trans* fatty acids in milk fat as a consequence of the ruminal biohydrogenation processes.

In dairy cows, some *trans* fatty acids (namely *trans* 10 C18:1 and *trans* 10, *cis* 12 C18:2) that originate from certain rumen biohydrogenation processes, are proposed as the cause of the milk fat depression syndrome (Bauman and Griinari, 2001), but this phenomenon has not yet been demonstrated in dairy sheep. Kitessa *et al.* (2001) reported that when sheep are fed fish oil supplemented diets (protected or unprotected), polyunsaturated fatty acids (PUFA) interfere with regular rumen metabolism, inducing a decrease of DMI and leading to the ruminal accumulation of *trans* C18:1 fatty acids.

Moreover, the Authors highlighted that ruminal biohydrogenation processes also induced the accumulation of other C18:1 fatty acid derivatives in the blood plasma, such as 10-hydroxy-stearic acid.

The inclusion of protected tuna oil in the ewe diet allowed an increase in the eicosapentaenoic acid (EPA, C20:5) content of milk, while the docosahexaenoic acid (DHA, C22:6) content of the milk remained unchanged (Kitessa *et al.*, 2001).

The general effect of calcium salt supplementation was an increase in the unsaturated fatty acids and a decrease of saturated ones in milk.

When calcium salts of palm oil were added to the ewe diets, an increase in the palmitic acid (C16:0) content of milk was also observed (Table 1), as a consequence of the high level of this fatty acid in palm oil (Pulina *et al.*, 1990; Todaro *et al.*, 1997; Rotunno *et al.*, 1998).

The inclusion of calcium soaps of olive oil in the ewe diets induced an increase of total octadecenoic acid content in milk (Perez-Alba *et al.*, 1997; Antongiovanni *et al.*, 2002; Table 2), but it is not yet clear if this increase is related only to the oleic acid content (cis 9 C18:1), or, as reported in dairy cows (Secchiari *et al.*, 2003), also to other positional and geometrical isomers of C18:1 that originate from ruminal biohydrogenation processes. Recently, an *in vitro* study on the ruminal biohydrogenation of oleic acid seemed to confirm the second hypothesis (Mosley *et al.*, 2002).

The mammary neo synthesis of medium chain fatty acids (MCFA) also seems to be inhibited by the calcium soaps of PUFA and this phenomenon may be described by a linear relationship (Schmidely and Sauvant, 2001):

% MCFA = 23.8 - 0.038 SC (g/d),
where % MCFA = milk content of MCFA and SC = amount of diet calcium soap.

No unfavourable effects on short chain fatty acid synthesis were reported when ewes were fed calcium soaps, especially with respect to butyric acid (C4:0) which has its origins in two known pathways that are independent of the inhibitable acetyl-coenzyme A carboxylase pathway. About

Table 1. Effect of the diet inclusion of calcium soap of palm oil on the fatty acid composition of milk in dairy ewes (Pulina *et al.*, 1990).

Fatty acids	Control	CSPO[1]	Significance
C4:0	1.35	1.33	
C6:0	1.44	1.05	
C8:0	1.71	1.07	
C10:0	6.05	3.58	*
C12:0	3.78	2.35	
C14:0	11.17	8.36	*
C16:0	29.84	34.19	*
C16:1	1.31	1.32	
C18:0	10.06	9.05	
C18:1 *cis + trans*	20.09	25.73	*
C18:2 n-6	4.92	5.38	
C18:3 n-3	0.51	0.43	

[1] CSPO = calcium soaps of palm oil.
* $P \leq 0.05$

Table 2. Effect of the diet inclusion of calcium soap of olive oil on the fatty acid composition of milk in dairy ewes.

Fatty acids	Control[1]	CSOO[1]	Significance	Control[2]	CSOO[2]	Significance
C6:0	3.78	3.69		2.26	1.31	
C8:0	2.86	2.93		2.73	1.38	
C10:0	10.34	9.35		9.57	4.16	
C12:0	6.11	5.27	*	5.69	2.66	*
C14:0	12.63	11.24	*	12.14	8.28	*
C14:1	0.32	0.26			-	
C15:0	1.30	1.14			-	
C16:0	27.27	23.90	*	24.09	21.57	*
C16:1	1.34	1.28		1.15	1.00	
C17:0	0.89	0.76			-	
C18:0	7.68	9.90	*	7.20	9.20	*
C18:1 *cis + trans*	19.74	24.54	*	13.96	25.09	*
C18:2 n-6	2.93	2.82		1.78	1.31	
Total CLA	1.11	1.24			-	
C18:3 n-3	1.16	1.11			-	
C20:0	0.55	0.57				

[1] Antongiovanni et al., 2002; CSOO = calcium soaps of olive oil;
[2] Perez-Alba et al., 1997; CSOO = calcium soaps of olive oil.
* $P \leq 0.05$

half of butyric acid arises directly from b-OH-butyric acid, while the remaining part is formed by a malonyl-CoA independent pathway by the condensation of acetyl units (b-reduction pathway) (Palmquist *et al.*,1993).

The total content of stearic acid (C18:0) and octadecenoic acid (C18:1) are also related to the amount of diet calcium soap according to a linear relationship (Schmidely and Sauvant, 2001):

% C18:0+C18:1 = 30.8 + 0.036 SC (g/d),

where % C18:0+C18:1 = milk content of C18:0 and C18:1 (cis and *trans* isomers); SC = amount of diet calcium soap.

In conclusion, the effects of fat supplementation on fatty acid composition of milk are only partially known and further studies are needed in order to better define the way to modulate the content of some fatty acids in milk that play an important role for human health, namely conjugated linoleic acid isomers, *trans* vaccenic acid (*trans* 11 C18:1), omega 3 PUFA and butyric acid. Although some evidence can be directly transferred from dairy cows, specific research may also take into account the metabolic and ethological peculiarities of the ovine species.

Conclusions

Although the number of studies about fat in dairy ewe feeding is not high, the results of the selected papers show that milk yield and fat content may be enhanced by lipid supplementation, especially when calcium soap of fatty acids are used, while the results regarding milk protein content are discordant. Regarding the fatty acid composition of milk, the general effect of calcium salt supplementation is an increase in the unsaturated fatty acids in milk and a decrease in the saturated ones. However, further studies are needed in order to better explain the effect of different fat source in dairy ewe feeding.

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