REVIEW ARTICLE



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ABSTRACT

The recent literature dealing with the effect of the diet on the quality of milk and meat fat is reviewed. Some aspects of the rumen metabolism of lipids are dealt with: lipolysis, bio-hydrogenation, synthesis of microbial fatty acids and inhibition mechanisms on fermentation. Firstly, the influence of forage is considered. Pasture is the best forage, better if high hill pasture, as compared to hay and silage: short chain fatty acids (SCFA) (shorter than C10) are increased, medium chain fatty acids (MCFA) (C12 through C16) are decreased, oleic (OA), linoleic (LA) and linolenic (LNA) acids are increased and so are the conjugated linoleic acid pool of isomers (CLA) and n-3 polyunsaturated fatty acids (n-3 PUFA). Secondly, the energy supplementation of diets with fats is looked at. Animal fats depress milk yield and SCFA, while OA is increased because of the enhanced activity of mammary Δ° desaturase. Fish oil depresses milk yield as well, but promotes CLA and n-3 PUFA. If animal fats are protected against rumen bacteria, milk yield and milk fat depression are avoided. Vegetable fats are richer in unsaturated fatty acids (UFA), thus more susceptible to the rumen bio-hydrogenation. As calcium soaps or inside whole seeds, plant fats are protected and CLA is increased. CLA is an important component of fat. In ruminants it comes from the desaturation of vaccenic acid (VA) both in rumen and udder; and the yield of VA depends on the diet quality. In conclusion, simple directions are given on how to improve the quality of animal fat by dietary means, without affecting yield.

Key words: Milk fat, Meat fat, Energy supplementation, CLA, Fatty acids

RIASSUNTO MIGLIORAMENTO DELLA FRAZIONE LIPIDICA DEGLI ALIMENTI DI ORIGINE ANIMALE ATTRAVERSO LA DIETA: ATTIVITÀ RUMINALE E PRESENZA DI ACIDI GRASSI TRANS E CLA NEL LATTE E NELLA CARNE

Viene passata in rassegna la letteratura recente riguardante gli effetti della dieta sulla qualità del grasso del latte e della carne. Si prendono in considerazione alcuni aspetti del metabolismo dei lipidi nel rumine: la lipolisi, la bioidrogenazione, la sintesi degli acidi grassi microbici ed i processi di inibizione delle fermentazioni. In primo luogo si considera l'aspetto dell'influenza dei foraggi. Il pascolo rappresenta la miglior forma di utilizzazione foraggio, soprattutto se pascolo di montagna, rispetto all'impiego di foraggi conservati sia attraverso la fienagione che l'insilamento: gli acidi grassi a catena corta (meno di C10) aumentano, quelli a catena media (da C12 a C16) diminuiscono, gli acidi oleico, linoleico e linolenico aumentano, come pure il pool di isomeri dell'acido linoleico coniugato (CLA) e gli acidi polinsaturi n-3. In secondo luogo si prende in esame l'effetto dell'integrazione energetica delle diete con i grassi. I grassi animali deprimono la quantità di latte prodotto e gli acidi a catena corta, mentre l'acido oleico aumenta a causa della maggiore attività della Δ° desaturasi mammaria. Anche gli oli di pesce hanno un effetto negativo sulla produzione di latte, ma promuovono quella di CLA e di acidi grassi polinsaturi n-3. Se i grassi animali vengono protetti contro l'attività dei batteri ruminali, si eliminano gli effetti depressivi sulla produzione del latte e del grasso del latte. I grassi vegetali sono più ricchi di acidi grassi insaturi e, pertanto, più suscettibili di subire la bio-idrogenazione ruminale. Sotto forma di saponi di calcio o all'interno di semi integrali, i grassi vegetali sono protetti e il CLA aumenta. Il CLA è un importante componente del grasso animale. Nei ruminanti proviene dalla desaturazione dell'acido vaccenico sia nel rumine che nella mammella; e la quantità di acido vaccenico prodotto dipende dalle caratteristiche qualitative della dieta. In conclusione, si elencano delle semplici informazioni su come migliorare la qualità dei grassi di origine animale attraverso la dieta, senza diminuirne la quantità prodotta.

Parole chiave: Grasso del latte, Grasso della carne, Integrazione energetica, CLA, Acidi grassi.

Introduction

All the foods of animal origin used by human beings over the centuries (meat from game and domestic animals, fish, eggs and milk) hold a more or less important lipid fraction. Only a few decades ago such fraction was looked at as a "rich" and hence desirable component. In fact the preference for fatty foods was due to the different style of life of our ancestors: work and transportation were not coped with by machines but by muscular power, so that a diet with a high energy concentration was necessary, since it was quite often quantitatively scarce. The incidence of cardiovascular disease as a consequence of hypercholesterolaemia was certainly lower, even because the average life span was shorter, due to other causes of death.

Nowadays the situation is completely upside down: the longer average life span makes our vascular system more susceptible to atherogenesis and thrombogenesis. Furthermore, the reduced and, in some cases, completely absent exercise, associated with hypercaloric diets, results in a greater and greater diffusion of people with problems of obesity, with undesirable consequences on their general health status. To end with a dangerous shortage of dietary fibre which favours the tumours of the lower gut.

The pressing question is then: is dietary fat dangerous? Of course the borderline between what is nutritionally beneficial and what is harmful depends on the quality and quantity of dietary fat intake. As far as the quantity is concerned, the individual energy daily requirements must be met. And, with reference to the quality, the composition of dietary fats of animal origin must be accounted for because there are beneficial fatty acids, some of them are even essential, and harmful ones. It is therefore necessary to upgrade the quality of animal fats by decreasing the harmful components and/or increasing the beneficial ones, but without changing the natural "identity" of the food product. This task may be achieved both through a genetic approach and through nutrition. As nutritionists, we shall deal with this latter aspect only.

Rumen metabolism of lipids

The studies of rumen metabolism of lipids pointed out that the two main processes which dietary lipids in contact with microbes are submitted to, are lipolysis and bio-hydrogenation (Harfoot, 1978; Palmquist and Jenkins, 1980; Jenkins, 1993). The first process refers to the release of free fatty acids from the esters of the diet lipid fraction, so allowing the subsequent bio-hydrogenation, i.e. the reduction of double bonds present along the carbon chain. Since the fatty acids, which are absorbed through the rumen epithelium or catabolised down to volatile fatty acids and carbon dioxide, are in very small amounts and since microbes are capable of synthesising fatty acids ex novo from precursor carbohydrates, lipids reaching the duodenum contain fatty acids both of dietary and microbial origin. The study of rumen lipid metabolism of the dairy cow is particularly important for two major

Acronym Name		Formula	Abbreviation		
AA	Arachidonic acid	CH3(CH2)3(CH2CH:CH)4(CH2)3COOH	C20:4 cis 5,8,11,14 n-6		
BA	Butyric acid	CH ₃ (CH ₂) ₂ COOH	C4:0		
CLA	Conjugated Linoleic acid	Pool of isomers	C18:2		
DHA	Docosohexaenoic acid	CH3(CH2CH:CH)6(CH2)2COOH	C _{22:6} cis 4,7,10,13,16,19 n-3		
DPA	Docosopentaenoic acid	CH3(CH2CH:CH)5(CH2)5COOH	C _{22:6} cis 7,10,13,16,19 n-3		
EPA	Eicosopentaenoic acid	CH3(CH2CH:CH)5(CH2)3COOH	C20:5 cis 5,8,11,14,17 n-3		
ETA	Eicosotetraenoic acid	CH3(CH2CH:CH)4(CH2)6COOH	C20:4 cis 8,11,14,17 n-3		
LAU	Lauric acid	CH ₃ (CH ₂) ₁₀ COOH	C12:0		
LA	Linoleic acid	CH ₃ (CH ₂) ₃ (CH ₂ CH:CH) ₂ (CH ₂) ₇ COOH	C18:2 cis 9,12 n-6		
LNA	α -Linolenic acid	CH3(CH2CH:CH)3(CH2)7COOH	C18:3 cis 9,12,15 n-3		
γ-LNA	γ-Linolenic acid	CH ₃ (CH ₂) ₃ (CH ₂ CH:CH) ₃ (CH ₂) ₄ COOH	C18:3 cis 6,9,12 n-6		
MA	Myristic acid	CH ₃ (CH ₂) ₁₂ COOH	C14:0		
OA	Oleic acid	CH3(CH2)7CH:CH(CH2)7COOH	C18:1 cis 9		
PA	Palmitic acid	CH ₃ (CH ₂) ₁₄ COOH	C16:0		
RA	Rumenic acid	CH3(CH2)5(CH:CH)2(CH2)7COOH	C18:2 cis 9 trans 11 n-6		
SA	Stearic acid	CH ₃ (CH ₂) ₁₆ COOH	C18:0		
VA	Vaccenic acid	CH3(CH2)5CH:CH(CH2)9COOH	C18:1 trans 11		

In order to facilitate the reader, a list of the acronyms used in the text is enclosed herein.

reasons: i) the possibility of controlling the antimicrobial effects of fatty acids in order to allow rations to be supplemented with fat sources without disturbances of rumen fermentation and of digestion processes; ii) the possibility of ruling bio-hydrogenation in order to control the absorption of specific fatty acids, capable of improving the performance or of upgrading the nutritional traits of milk.

Lipolysis

A short time after intake, dietary lipid esters are hydrolised by microbial lipases which cause the constituting fatty acids to be released (Figure 1). One of the bacteria well-known for its lipolytic action is *Anaerovibrio lipolytica*, producing a membrane esterase and a lipase (Harfoot, 1978). Lipase is an extra-cellular enzyme assembled inside particles equipped with a membrane made up of proteins, lipids and nucleic acids (Jenkins, 1993). The lipase hydrolyses completely tri-glycerides down to free fatty acids and glycerol, leaving small amounts of mono- and di-glycerides. Glycerol is then fermented to propionic acid. Even though the lipase activity of *Anaerovibrio lipolytica* is intense as compared to that of other non-lipolytic microbes, its esterase activity is weaker. By using p-nitrophenylpalmitate, 74 different bacterial strains capable of hydrolysing the ester bonds were identified (Fay *et al*, 1990). Some of these strains, among them *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens*, showed a low hydrolysis activity, whereas other strains, endowed with esterase activity, not necessarily resulted capable of hydrolysing lipid esters. Only a few out of the numerous rumen bacteria with esterase activity (including 30 strains of *B. fibrisolvens*) are capable of hydrolysing long chain fatty acids (Jenkins, 1993).

In addition to the enzymatic hydrolysis of triglycerides, fatty acids may also come from the hydrolysis of galactolipids and phospholipids operated by different galactosidases and phospholipases (phospholipase A, phospholipase C, lysophopsholipase and phosphodiesterase), produced by rumen microbes (Jenkins, 1993). Kemp (cited by Palmquist and Jenkins, 1980) could identify a good five micro-organisms, including *Ruminococcus albus*, capable of isomerising fatty acids with more than one double bond. Figure 1. Lipolysis and bio-hydrogenation.



Bio-hydrogenation

The half-life duration in the rumen liquor of free unsaturated fatty acids is relatively short because of their quick hydrogenation to the saturated form operated by microbes. The percentage of hydrogenated PUFA was estimated to be between 60 and 90%. Even though the subject is still debated, the process appears utilised by microbes in order to protect themselves from the toxic effects of unsaturated fatty acids. This hydrogenation process contributes only for a small part to the recycling of metabolic hydrogen because only 1-2% of it is utilised in this way (Jenkins, 1993). In the case of unsaturated acids with one of the double bonds in the position cis 12 (e.g. LA and α -LNA), the first step of the bio-hydrogenation process consists in an enzymatic isomerisation reaction converting the cis 12 bond into a trans 11 one (Figure 1). Generally speaking, this isomerase should work only in the presence of a free carboxyl function and, in the particular case of polyunsaturated acids, in the presence of the isolated *cis* 9, *cis* 12 diene. The presence of a free carboxyl makes lipolysis as a pre-requisite for the following reduction; in fact lipolysis may be considered as the "rate determining step" of the whole process, that is the step which determines its kinetics. The passage of a very small amount of polyunsaturated fatty acids through the rumen wall could, therefore, be due to a missed lipolysis. After the trans 11 bond is formed, a microbial reductase operates the hydrogenation in the cis 9 position. The amount of VA which is reduced to SA is influenced by both the rumen conditions and the concentration of LA which irreversibly inhibits the process (Harfoot et al., 1973). Moore et al. (1969) suggested that large amounts of unesterified LA may block the second step of bio-hydrogenation, but this would not occur with LA in the esterified form. Gerson and King (1985) demonstrated that the dietary fibre/starch ratio may have an influence on the lipolysis and bio-hydrogenation rate in the sheep because, most probably, the activity of cellulolytic bacteria is disturbed. Actually, if starch (barley) is gradually substituted for fibre in a diet, the rate of both processes slows down with the consequent accumulation of C_{18:1}. By incubating LA [¹⁴C] and sucrose with rumen fluid from ewes fed highly

fibrous rations, the in vitro bio-hydrogenation rate increases more than 40% with a concentration of the sugar of 0.5%, but with starch the lipolysis rate increases and the bio-hydrogenation remains unaltered. In short, several dietary factors may affect the composition and the proportion of rumen lipids. One of these factors, as already mentioned, is the percentage of grains which appears to particularly influence the bio-hydrogenation process. As a matter of fact, a decrease of 59 and 63% was observed for the reduction processes of LA and LNA, respectively, with low fibre diets as compared to diets characterised by high forage/concentrate ratios. This effect appears to be linked to the decrease of cellulolytic bacteria which are responsible for the lipolysis process, a necessary pre-requisite to bio-hydrogenation. Hence, this kind of diet might increase the amount of feed lipids succeeding in trespassing on the rumen wall without undergoing hydrogenation; the greatest benefit comes from both OA and LA, the fatty acids represented at a greater extent in cereal grains. The effects of rations with a high level of cereal grain are exerted also on the composition of the portion of feed lipids escaping the rumen transformations. In fact, the decreasing of lipolysis could favour the formation of trans isomers of C18:1 in the rumen, as observed in cows fed diets rich in maize grain (Palmquist and Schanbacher, 1991). Among the other dietary factors which depress lipolysis and bio-hydrogenation may be mentioned: i) a low level of dietary nitrogen; ii) the use of mature forage and iii) the use of too finely ground feeds. In this last case, the size of feed particles is of particular importance because it affects the adherence of bacteria upon the surface and increase the transit rate through the rumen barrier, so shortening the time of exposition to the activity of microbes.

Lastly, the quantity and type of fat added to the diet are other factors capable of affecting the transformation of lipids in the rumen. Fats with a high content of LA, like soybean oil, inhibit the processes of reduction to SA, so favouring the accumulation of intermediate compounds such as *trans* isomers of $C_{18:1}$ and, mainly, of VA. This is even more evident when LA is administered as a free acid (Moore *et al.*, 1969). The most common approach to let unsaturated fatty acids pass to the duodenum escaping the rumen consists in protecting the lipid source. One of the oldest technologies is the encapsulation with formaldehyde treated protein, which results in a significant increase of unsaturated acids at the duodenum level and, consequently, in milk. Another well-known strategy consists in the inclusion in the diet of full fat whole oil seeds so that the integument may protect the inside oil. It has been demonstrated that chewing partially defeats this kind of protections and the inner fat becomes accessible to rumen microbes (Murphy et al., 1987; White et al., 1987; Keele et al., 1989). Calcium salts appear to resist to biohydrogenation because they might limit the availability of free carboxyl groups. In any case, the efficacy of protection is strictly linked to the chemical nature of the mixture of fatty acids used to produce the soap: the higher the unsaturation level of molecules, the easier the dissociation of the salt in the rumen liquor and the following bio-hydrogenation because of the higher value of the salt hydrolvsis constant (Kid). Due to this reason calcium salts of palm oil showed a higher resistance to rumen transformation than other soaps like calcium linoleate (Klusmeyer and Clark, 1991; Wu et al., 1991; Wu and Palmquist, 1991; Fotouhi and Jenkins, 1992). Another approach to limit the release of free carboxyl functions consists in changing them into amides. In this latter case the resistance to microbial attacks seems to be due to the steric encumbrance of the substituting groups bound to nitrogen (Jenkins, 1993). If methionine is used as the amine group donor, the dual purpose of protecting unsaturated fatty acids and increasing the duodenal flow of this amino acid may be achieved. (Langar et al., 1975; 1978).

Synthesis of microbial fatty acids

The lipid fraction of rumen bacteria is about 10-15% of the dry matter and is originated partly from degraded feeds and partly from *ex novo* syntheses which take place within the microbial cells. The contribution of each of these pathways depends on the fat content of the diet and on the bacterial species present in the rumen liquor (Jenkins, 1993). As a matter of fact, if the dietary lipid level is high, the transportation of lipids into

the bacterial cell is favoured and tiny droplets are formed in the cytoplasm. On the contrary, the ex novo synthesis of fatty acids leads prevalently to the formation of SA and PA in the ratio 2.1/1 (Bauchart et al., 1990). Furthermore, it must be noted that the microbes do not store triglycerides, but phospholipids and, for a small part, as free non-esterified fatty acids (NEFA) (Viviani, 1970). Some specific studies on the biosynthesis mechanisms revealed that the absorption of ¹⁴C marked acetate and glucose by rumen bacteria leads to the synthesis of non-branched fatty acids with an even number of carbons, whereas the absorption of ¹⁴C marked propionate and valerate, leads to the synthesis of long chain fatty acids with linear chain but with an odd number of carbon atoms. Instead, by utilising iso-butyrate, iso-valerate and 2methyl butyrate as the precursors, the synthesis of branched acids in the iso- and anteiso- forms is observed. In bacteria, this latter class of lipids is about 20% of total fatty acids and about 30% of fatty acids contained in phospholipids. The anteiso isomer C_{15:0} is the most represented one, but it is possible to find small amounts of the iso-anteisoforms of acids with chain length from 14 to 17 carbon atoms. The mono-unsaturated acids (MUFA), which account for about 15-20% of microbial acids, are synthesised anaerobically (figure 2). Through this pathway, β -hydroxydecanoate is de-hydrated in β, γ , so forming a double bond in position 3 and cis geometrical isomerism instead of being dehydrated in α,β , which would lead to the *trans* 2 isomer. With the double bond in cis 3, the subsequent reduction operated by C₁₀ enoyl reductase is not possible, so allowing the chain elongation up to C_{16:1} and C_{18:1}. This latter acid may be also formed by desaturation operated by a desaturase present in the rumen liquor (Jenkins, 1993). Cyanobacters only, present in very small percentages, synthesise the polyunsaturated acids (PUFA); the presence of such acids at the rumen level is therefore mainly due to feeds rather than to the ex novo microbial synthesis. On the contrary, protozoa incorporate long chain fatty acids (LCFA) under the form of membrane lipids.

A simplified classification subdivides rumen bacteria in three groups: those present in the liquid phase (LAB); attached to the feed particle (SAB); dispersed (Bauchart et al., 1990). It is quite interesting to note that in the rumen liquor of cows fed fat enriched diets the concentration of total lipids is increased (mainly phospholipids, galactolipids and free fatty acids) and that the contribution of SAB is the most important one. The mechanism with which SAB bacteria are capable of incorporating the fatty acids of feeds has been discussed for a long time. The most likely hypothesis is that the droplets of free fatty acids (FFA) are adsorbed on the surface of the bacterial cell and subsequently transported inside the cell. In fact, droplets of fat containing free fatty acids have been observed inside the cytoplasm of bacteria incubated in the presence of lipids, so confirming such hypothesis. As a further confirmation, if the rumen liquor is incubated with soy bean oil rich in LA ($C_{18:2}$ n-6), this fatty acid is preferentially absorbed by SAB bacteria after hydrolysis and can be found as a free acid within the droplets prior to the occurrence of any reduction. If the molecules were simply immobilised on the outside bacterial surface, their double bonds would certainly have undergone hydrogenation. On the contrary, LAB bacteria absorb preferably branched fatty acids (iso- and anteiso-) which render the membrane cell more deformable.

Lipid balance in the rumen

The feed dry matter ingested by ruminants contains on average about 4% ether extract (crude fat) and only 40% of which in forages and about 70% in concentrates is represented by fatty acids (Palmquist and Jenkins, 1980). The amount of acids lost in the rumen is negligible, as some works, which studied the absorption of long chain fatty acids (LCFA) through the rumen wall and their degradation down to volatile fatty acids (VFA) and CO₂, could demonstrate. Wood et al. (1963), after infusing marked LA into a sheep rumen with the reticulo-omasal orifice which had been tied, found out that the amount of VFA degraded was less than 1% and that the amount of radioactive LA present in plasma after 48 hours was less than 0.3%. Yet, some metabolic pathways that would lead to the loss of fatty acids from the rumen liquor have been hypothesised. In fact, OA is absorbed by rumen epithelial cells as much as

31.5% and transported into plasma as much as 8.2% (Jenkins, 1993). On the contrary, PA is rapidly metabolised to ketones and transformed to C_{15:0} via α -oxidation and to C_{13:0} and C_{11:0} via β -oxidation (Jesse et al., 1992). Furthermore, more than 90% of fatty acids shorter than 14 carbon atoms would be absorbed through the rumen wall. It was observed that when feeds pass from the mouth to the duodenum the possible loss of lipids occurs more frequently with fat enriched diets than with diets poor in fat. On the base of numerous studies, the contribution of microbial syntheses to the amount of lipids reaching the duodenum has been estimated to be 15 g per kg dry matter fermented in the rumen. The amount of fermented organic matter and of lipids in the diet should, in fact, be the only factor influencing the lipid syntheses of microbes. In conclusion, on average 87% of ingested fatty acids may be found in the duodenum; the small loss is often made up for ex novo microbial syntheses, with a little benefit while passing the rumen. The causes of losses may be attributed to the lipid metabolism of rumen epithelial cells and to microbial degradation (Jenkins, 1994).

Effects of lipids on rumen fermentation

The addition of lipids to ruminant diets may exert negative effects upon rumen fermentation by decreasing the digestibility of non lipid energy sources. An amount of fat of less than 10% in the ration may depress the rumen degradation of structural carbohydrates as much as more than 50% (Ikwuegbu and Sutton, 1982; Jenkins and Palmquist, 1984). And this comes along with a lesser production of methane, hydrogen, volatile fatty acids (VFA) and with a decrease of the acetate/propionate ratio (Ikwuegbu and Sutton, 1982; Chalupa et al., 1984; Boggs et al., 1987). Protein metabolism may be affected by the fatty supplementation of diets as well. In fact, the infusion of linseed oil in the rumen of sheep or likewise the addition of maize oil or lecitine to rations for dairy ewes put in evidence a decrease of rumen degradability of proteins, a lowering of the concentration of ammonia and a rise of the nitrogen flow to the duodenum (Jenkins, 1993).

The importance of fat supplemention to rations of dairy cows has induced researchers to verify the

Figure 2. Microbial synthesis of saturated and mono-unsaturated fatty acids.





Figure 3. Fate of unsaturated fatty acids in the rumen.

effects of a great variety of fat supplements on rumen fermentation. Usually the influence exerted on rumen metabolism depends on a few basic differences of the lipid structure. One of the structural aspects is the degree of unsaturation: in fact polyunsaturated acids (PUFA) inhibit fermentation at a higher extent than saturated acids (SFA) (Palmquist and Jenkins, 1980; Chalupa et al., 1984). Also the presence of a free carboxyl group seems to be of a certain importance in this respect: calcium salts of fatty acids and derivatives of carboxylic acids like amides, triglycerides and long chain alcohols inhibit fermentation at a lesser extent than what free fatty acids can do. Hence, unesterified unsaturated fatty acids constitute the lipid fraction which exerts the strongest influence on fermentation processes. The concentration of the UFA free in the rumen is ruled by both quantity and quality of fat in the diet, by the extent of lipolysis, by bio-hydrogenation and by the formation of carboxylated salts (Figure 3). As already outlined, high fat concentrations in the diet mean high amounts of total lipids in the rumen, nevertheless the possibility that the pool of UFA may be

increased depends on how efficient are the lipolysis and bio-hydrogenation processes or on the possible formation of salts. Generally speaking, the rate of lipolysis is sufficient to hydrolise in a short time the great majority of triglycerides, unless some studies provide evidence that lipolysis and bio-hydrogenation are substantially modified by ageing of forages, by the level of nitrogen and by the size of feed particles in the rumen.

The rate of bio-hydrogenation in vitro may vary with the concentration of substrates in the culture medium, with the kind and age of the inoculum, with the presence of some co-factors in the rumen liquor (Kellens *et al.*, 1986).

The formation of calcium salts depends on the solubility of dietary calcium, on the level of lipids in the ration, on rumen pH, on the degree of saturation and on the length of the carbon chain of acids.

Inhibition mechanisms

As already mentioned, four theories have been proposed in order to explain how lipids interfere with rumen fermentation: i) the physical assembling of fibre with fat makes the bacterial attachment on the surface of feed particles difficult; ii) the microbial population is modified due to the toxic effect of lipids; iii) the activity of microbes is inhibited due to surface effects exerted by fatty acids on the cell membrane of bacteria; iv) the concentration of cations is reduced as a consequence of the formation of insoluble complexes with long chain fatty acids (LCFA), so causing a variation of the ionic activity and hence of pH, which has an influence on microbial population (Dewendra and Lewis, 1974). The first two theories have received the major attention. As far as the first theory is concerned, it has been observed that pure cultures of bacteria are capable of absorbing more than 90% of fatty acids before feed particles are added; after that, more than 60% of fatty acids are associated with the particles. Hence, this theory would explain the slow down of fermentation with the formation of a lipid layer which would inhibit the degradation of cellulose by wrapping the feed particles. The physical contact between microbes and feed particles is in fact the necessary condition for cellulolytic enzymes to be active and the lipid layer would prevent the contact (Jenkins, 1993). Even though bacteria are attached to the surface of the feed particle, the depressing action of fats on cellulases occurs all the same. It has been observed that the presence of free fatty acids in a mixture of rumen cellulases and carboxy-methylcellulose weakens the enzyme-substrate linkage, so reducing the cellulase activity. To support the second theory is the fact that the addition of fatty acids to pure cultures of rumen bacteria results in the inhibition of bacterial growth, so demonstrating the direct anti-microbial effect of lipids. The anti-microbial effects of lipids in the rumen exhibit several similitudes with the cyto-toxic action of fatty acids on the membrane functions of euchariotic cells (Borst et al., 1962; Jenkins and Futouhi, 1990). Long chain fatty acids (LCFA) attack the double lipid layer of biological membranes quite easily because of their hydrophobic and anphiphylic nature. Ten different pathways through which fatty acids can alter the functions of biological membranes have been identified, at least (Gutknecht, 1988). One of the hypotheses is based upon the likelihood of interactions of these compounds with the lipid component of mem-

branes, whereas another hypothesis suggests the possible formation of bonds with membrane proteins (Gruber and Low, 1988). Such mechanism is supposed to occur in the membrane of rumen microbial cells as well. The free carboxyl function plays a fundamental role in the reactions involved in such interactions, so explaining what hypothesised above about the minor influence on rumen fermentation of salts and of acidic derivatives.

Conjugated Linoleic Acid (CLA)

During the last years medicine drew people's attention on the importance of prevention as the winning strategy to fight the severest pathologies affecting our society. Nutrition is surely one of the most efficacious means, because substances capable of fortifying the immune system, sometimes acting directly on the cause of disease, are introduced into the organism along with food. Milk, apart from its nutritional traits, contains substances which have beneficial effects on human health and is, therefore, considered essential to a correct nutrition. In particular, in milk are present vitamin A, vitamin E, β -carotene, sphingomyelins, butyric acid and CLA, all with a strong antitumour effect (Parodi, 1999). The lipid fraction of milk fat is therefore characterised by the presence of some fatty acids endowed with particular beneficial properties. CLA is undoubtedly the most characteristic one in this respect. It is synthesised during rumen bio-hydrogenation and partly in the mammary gland. In the last decade CLA aroused much attention in our scientific world because several in vivo and in vitro studies put in evidence, besides its anti-carcinogenic activity, also antiatherogenic, anti-obesity, anti-diabetes and immune-stimulating properties (McGuire and McGuire, 1999). Since the food products from ruminant animals, milk in particular, are those naturally richer in CLA, attempts are being made to further enrich its content by means of nutritional strategies, in order to get safer and safer foods to the consumer.

Biosynthesis of CLA in ruminants

CLA is present in milk and meat of ruminants and is formed through two metabolic pathways. The

Figure 4. Rumen microbial bio-hydrogenation of linoleic acid to trans conjugated linoleic (CLA) and trans octadecenoic acids with shift to *trans* 10.



octadecanoic (stearic)

first one is the rumen bio-isomerisation of LA. The second one is the desaturation of VA in the mammary gland, coming from the rumen (Figure 4).

As already widely described in the preceding sectors, lipids reaching the rumen are hydrolised by bacteria and fatty acids are set free from triglycerides and phospholipids. Such reaction is an essential event because the following saturation of unsaturated acids (UFA) may happen. The only bacterium held capable of carrying out biohydrogenation was for many years Butyrivibrio fibrisolvens (Kepler et al., 1967). But quite recent studies could identify other microbes capable of saturating double bonds (Harfoot and Hazlewood, 1988). Studies carried out on pure cellular cultures demonstrated that the whole hydrogenation process is not performed by a single micro-organism, but is co-ordinated by a pool of bacteria which manage the various steps. Hence, bacteria may be divided into two groups: group A which saturates LA and LNA to VA and group B which concludes the sequence of hydrogenations by reducing VA to SA (Harfoot and Hazlewood, 1988). The synthesis of CLA in the rumen is shown in Figure 4. The initial step is the isomerisation of *cis* 9, *cis* 12 C₁₈₋₂ (LA) to cis 9, trans 11 C_{18:2} (RA), one of the CLA isomers. This step is catalysed by the enzyme linoleic isomerase which doesn't take advantage of cofactors and acts on double bonds located in the centre of the carbon chain, far from the activating functional groups. The enzyme is bound on the bacterial membrane and is very selective because only dienes of the type cis 9, cis 12 on the carbon chain of fatty acids with a free carboxyl function are recognised. The second step is a saturation converting RA into VA (trans 11, C18:1). In vitro studies with marked LA incubated in rumen liquor demonstrated that this second step is quite rapid. On the contrary, the following saturation of VA to SA is much slower so allowing its accumulation in the rumen and its passage into the blood plasma (rate determining step). The formed VA, when absorbed and transported to the mammary gland, may be reconverted into RA by the action of a Δ^9 desaturase. It is well known that possible modifications of the microbial population due to changes of the fermentation conditions, like for instance the lowering of pH, may modify the acidic

composition of milk fat. In the case of CLA synthesis, a diet for beef cattle, characterised by a high level of concentrate and a low level of fibre, may divert the saturation of LA towards the synthesis of other trans decenoic isomers. In this case trans 10 C_{18:1} is formed in the place of VA (shift effect) during the fermentation process (Griinari et al., 1998). The proposed mechanism involves the cis 9, trans 10 isomerase that would lead to the formation of trans 10, cis 12 CLA as the first intermediate of the conversion of LA. In the following step, the saturation of the bond in the 12 position leads to trans 10 C_{18:1} (Figure 4) (Griinari, 2001). Trans 10, cis 12 CLA seems to be exclusively synthesised in the rumen because the existence of a Δ^{12} desaturase in the mammary gland was not demonstrated. The saturation of LNA takes place in a manner very close to bio-hydrogenation of LA. The C_{18:3} isomer predominant in food is a-LNA. Its isomerisation produces cis 9, trans 11, cis 15 C_{18:3} from which, again by saturation, trans 11 C_{18:1}. The fermentation pathway to the less common α -LNA (cis 6, cis 9, cis 12) is absolutely analogous (Hartfoot and Hazlewood, 1988; Griinari and Bauman, 1999).

Studies carried out by Buccioni (2002) on the evolution of the concentration of the major C_{18:1} isomers and of CLA isomers in the rumen fluid during in vitro fermentation processes confirmed the tight link of the precursor-derivative type existing between LA, RA and VA (Figure 5), so putting in evidence that the cis 9, trans 11 isomer is not only the pre-eminent one, but is also the fastest to be formed and saturated. The supplementation with calcium soaps of olive oil fatty acids or with full fat extruded soybean induced the shifting of acidic composition of rumen liquor towards the saturated fractions. The same trend was observed with reference to the acidic composition of milk fat produced by cows fed the same diets which had been tested in vitro. Interesting peculiarities of the syntheses and of the changes of fatty acids operated by the microbes emerge from the analysis of the acidic composition of incubated rumen liquor (Secchiari et al., 2003). The administration of calcium soaps of olive oil and of full fat soybean (Table 1) induced an increase of the monounsaturated fraction in both theses, but only soybean promoted the rise of PUFA (Table 2). This behaviour may be explained with the higher content of OA and LA present in the rumen fluid, incubated with samples of diets, fat supplemented at a higher extent with reference to the control diet (p<0.01). In fact, while soybean is rich in LA, susceptible to be partially saturated in the rumen environment to mono-unsaturated intermediates, olive oil calcium soaps are naturally rich in oleic acid. The behaviour of olive oil in the rumen was quite interesting: it would appear to be transformed into other C_{18:1} isomers instead of being saturated to SA. This would confirm what reported by Selner and Schultz (1980) who found an increase of trans C18:1 acids in milk fat from cows fed a diet supplemented with pure OA. Since the mammary gland picks up long chain fatty acids directly from the blood stream, what was found in the milk of cows fed the same diets which were tested in vitro may be considered a direct consequence of isomerisations in the rumen.

Effects of the diet on the composition of milk and meat from ruminants

Quite a high number of studies, all very recent,

on the influence of the diet on the composition of milk fat are available in the literature. Chilliard *et al.* (2001; 2002) have recently put in evidence that it is possible and relatively easy, rapid and efficacious, to affect milk fat quality by changing the composition of the diet at the farm level and in a reversible manner. When talking of milk fat quality, we must refer to:

- the organoleptic characteristics, mostly due to the presence of short chain free fatty acids;
- the melting point and consistency (spreadability) of butter, again due to a relatively high presence of saturated short chain acids and unsaturated acids;
- the nutritional effects on the consumer's health (hypocholesterolemic, anti-atherogenic, anti-thrombogenic, anti-carcinogenic and anti-obesity).

Whereas, with reference to meat, we must look at the acidic composition of both the polar fractions (lipids of the cell membrane) and the apolar fractions (storage fat) of the intra-muscular fat.

Let's begin with considering the effect of the nature of forages. The studies on this subject are actually few, because the investigations carried out so far have dealt with the effect of the addition





of animal fat, fish oils and vegetable oils to typical forage-concentrate diets.

The green forages of our temperate regions contain about 1-3% fatty acids, maximally in Spring and Autumn. More than a half of such acids are represented by α -LA. In the tropical forages the percentage of LNA represents 15-40% of total FA (Chilliard *et al.*, 2001).

The acidic composition of forage plants is, in any case, strongly influenced by the species they belong to and, also, by the frequency of utilisation of the herbaceous surface (Dewhurst et al., 2001). Furthermore, it is relatively easy to affect the milk fat quality at the farm level simply by changing the forage/concentrate ratio. Table 3 clearly depicts these aspects. In the first place, it is evident that there is a great difference between diets based on forage only and diets with concentrate. With 100% forage the quality of milk fat is decisively better: lower concentrations of LAU to PA known as atherogenic and thrombogenic acids (Ulbricht and Southgate, 1991) and higher amounts of unsaturated C₁₈ and CLA acids, the beneficial ones. In the second place, the nature of forage is a critical factor: there is a difference in favour of high hill pasture which results in a higher amount of C18:1, but especially in twice as much CLA, a desired and desirable component while the concentration of MA, the most harmful fatty acid

(Ulbricht and Southgate, 1991), is not different between pastures, but much lower than in the milk of cows fed concentrates. These findings have been recently confirmed by Lock and Garnworthy (2003), who verified that the milk fat of cows fed fresh grass during the Summer contained significantly greater amounts of CLA and short chain fatty acids, due to the increased Δ^9 desaturase activity in the mammary gland. LA and LNA deserve a special comment because the former is the precursor of RA, the most important CLA formed in the rumen (Figure 4), and the latter, three to fivefold with pasture, is the precursor of n-3 fatty acids formed in the mammary gland from LNA (Figure 6). And both CLA and n-3 acids are well-known beneficial factors (Parodi, 1997; 1999; Andriamampandry et al., 1999; Mantzioris et al., 2000; Hornstra, 2000). As a matter of fact, the amounts of the poly-unstaurated acids LA and LNA are small in any case in milk fat, so confirming the intense activity of microbial bio-hydrogenation within the rumen. The relative innocuity of SA makes us look at its higher content in the milk of cows fed pasture only, with tranquillity.

The apparent superior quality of green fresh forage over herb silage in terms of unsaturated/saturated ratio may be due to the presence of concentrate in the diet, but more likely to possible modifications occurred within the mass during matura-

		Diets			
Feed ingredient	A	В	С		
Alfalfa hay	28	29	32		
Maize silage	31	29	22		
Concentrate(*)	-	-	42		
Full fat soybean	-	14	-		
Maize meal	27	28	-		
Soybean meal	13	-	-		
Wheat straw	-	-	4		
Calcium soaps of olive oil	1	-	-		

Table 1.Ingredient composition of diets tested *in vitro* and fed to dairy cows
(% of DM) (Secchiari et al. 2003).

(*) Concentrate was a commercial product for dairy cows, prepared according to the "Grana Padano" regulation 1990. Composition: maize meal, barley meal, roasted soybean meal, maize gluten feed, full fat soybean meal, wheat middlings, partially dehulled sunflower seed meal, wheat bran, carob meal, partially dehulled cotton seed meal, cane molasses, CaHPO4.2H2O, CaCO3, NaHCO3, NaCl, MgO, DL-methionine. tion from undesired fermentation (Chilliard et al., 2001). However, maize silage is a particular forage, different from all the other silages, mainly because of its high concentration of LA. In fact, maize grain, normally present in the silage mass as much as about 30%, contains up to about 60% LA (on the ether extract basis). When maize silage exceeds 60% of the ration dry matter, experimental data of the literature (Chilliard et al., 2001) indicate that the acids from C_6 to C_{12} and LA are favoured at the expenses of PA, SA and LNA. Furthermore, in the milk fat of animals fed diets with more than 60% maize silage, VA and the major CLA, RA may be found in small amounts, less than half of those found in the milk fat of cows fed other green forage crops.

As far as hay making as a preserving technique is concerned, hays are reported by the literature to decrease the concentration of long chain fatty acids and, in particular, of LNA, with the consequent depression of the amount of n-3 acids in milk (Chilliard *et al.*, 2001).

The effect of pasture feeding on intramuscular fat is important as well (Table 4) because it strongly influences the acidic composition both of medium chain and of long chain fatty acids. In particular, PA, a well-known atherogenic factor, is decreased, whereas CLA and α -LNA are remarkably increased (CLA more than twofold). As a consequence the n-6/n-3 ratio changes in favour of n-3, towards an optimal value for the consumers' requirements. With reference to those particular aspect, the literature reports that Friesian bullocks fed rations based on green herbage showed a greater content of n-3 PUFA (LNA and EPA) in their meat fat than bullocks fed on hay and cereals (Geay *et al.*, 2001).

The preferential absorption of LNA and its n-3

of fermented rumen fluid (Secchiari et al. 2003).						
	Diet A (soap)	Diet B (soy)	Diet C (control)	A+B <i>vs</i> C	A <i>vs</i> B	
MCFA	25.87	20.72	20.97	**		
LCFA	21.89	39.99	25.06	**	**	
SFA	29.89	29.89	29.02			
MUFA	17.44	16.10	8.44	**		
PUFA	9.04	18.16	8.05	**	**	
Single fatty acids:						
C10:0	0.75	0.76	0.76			
C _{12:0}	5.42	3.21	3.27	*	**	
C14:0	3.30	1.97	2.09			
C16:0	15.61	13.90	14.25			
C18:0	4.97	7.51	8.58	**	**	
C18:1 trans 11	4.50	5.44	3.60		**	
C18:1 cis 9	11.97	9.87	4.64	**	**	
C18:2 cis 9 cis 12	7.96	15.89	6.77	**	**	
CLA cis 9 trans 11	0.35	0.49	0.21	*		
Total C18:1 cis	12.49	10.32	4.82	**		
Total C18:1 trans	4.60	5.52	3.61	**	*	
Total CLA	0.58	0.76	0.38			

Table 2.	Effect of incubated diets on the acidic composition (g/100 g lipids)
	of fermented rumen fluid (Secchiari et al. 2003).

Legenda: MCFA = medium chain fatty acids ($C_{12} - C_{16}$); LCFA = long chain fatty acids (> C_{16});

MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids. * = P<0.05; ** = P<0.01.

metabolites (EPA, DPA, DHA), so important to the consumers' health, was confirmed also by Enser *et al.* (1999) by means of feeding trials of beef calves and lambs fed green herbage. On the contrary, diets based on cereals lead to an increase of n-6 PUFA in the depot fat. Neutral lipids, mostly triglycerides, present in the muscular tissue embody preferentially PUFA such as LA and LNA, whereas the polar ones, like the phospholipids, are less selective and may contain also unsaturated fatty acids such as C₂₀₃, ETA and DPA. The same occurs with the meat of lambs, in which LA and LNA are increased with diets based on green herbage (Bas and Sauvant, 2001).

In conclusion of this sector dedicated to forages, it can be observed that, if one must look at the quality of milk and meat and not only at the quantity, in the right seasons when available, the use of green herbage, better if hill pasture, with the minimum contribution of concentrates, is a must. The milk fat produced is richer in very short chain fatty acids (< C_{10}), poorer in atherogenic and thrombogenic factors (C_{12} , C_{14} , C_{16}), richer in unsaturated acids, in CLA and n-3. Exactly the same can be said about meat quality.

Effects of diet supplementation with fats.

Animal fats.

Animal fats have been broadly used with practically all the categories of farm animals, but mostly with high yielding dairy cows, during the so called "peak of lactation", when energy requirements are particularly high. Recently, in consequence of the well-known problem of BSE, all feeds of animal origin are looked at as potentially harmful and also the fats of animal origin are no longer so popular. Nevertheless, animal fats have been used and still may be used and therefore the subject is worth some comments, starting from the complete review by Chilliard *et al.* (2001).

Tallow is the most widely used fat with dairy cows. Ruminant fat is particularly rich in PA (23-27%), SA (14-29%) and OA (36-50%). Bovine tallow may be administered either as such, unprotected against rumen bio-hydrogenation, or emulsified and capsuled within a layer of formaldehyde denatured proteins for protection. Protection is in no case complete, reaching 65% as the maximum value, obtained in vivo.

If unprotected fat is administered over 7% of the diet dry matter, the concentration of milk fat falls down (Palmquist and Jenkins, 1980). The reason for that is to be found in a modification of the rumen environment and, in accordance with some authors, in the formation of specific fatty acids capable of negatively affecting the expression and/or the activity of lipogenic enzymes at the mammary tissue level (Bauman and Griinari, 2001). Thus, the depression of milk fat could depend on a marked reduction of de novo synthesis of short and medium chain fatty acids (C₆ - C₁₆) and of LA, whereas OA should be increased. Because of the enhanced activity of Δ^9 desaturase, if OA replaces short chain acids on the sn-3 position of triglycerides, fat fluidity remains unmodified. PA is not influenced.

If rumen-protected fat is administered (emulsified and captured within a layer of formaldehyde denatured proteins), the response is different. In fact, an increase of the concentration of milk fat occurs together with an increase of milk yield. The qualitative modifications regard again the short and medium chain fatty acids $(C_6 - C_{16})$, that decrease and the C18 OA and SA, that on the contrary increase. OA comes partly from direct absorption and partly from the activity of Δ^9 desaturase on SA, as clearly demonstrated by studies with SA supplementation. LA and LNA appear not to be affected by fat supplementation. In conclusion, it was observed that the administration of protected tallow promotes effects which are comparable to those induced by the mobilisation of adipose body reserve.

Fish oils are rich in polyunsaturated long chain acids (20-22 carbon atoms). Among them the already cited n-3 EPA and DHA stand out for quantity and importance. The composition of fish oils is in any case extremely variable: e.g. EPA may vary between 4 and 32% and DHA between 2 and 25% (Moffat and McGill, 1993). Even if the hypothesis is still uncertain, it is possible that an uncompleted hydrogenation of these two acids may occur within the rumen, preferably of EPA, so losing a great part of them. As a matter of fact, experimental data on the efficiency of transportaFigure 6. Genesis of n-3 polyunsaturated fatty acids.



tion of n-3 from mouth to udder are quite disappointing: only about 2% for EPA and 4% for DHA. However, such results are probably due to the preferential inclusion of these fatty acids within phospholipids, which are less than 1% in milk. Generally speaking, the supplementation with fish oil induces a remarkable depression of milk fat. Some hypotheses have been proposed to explain such depressive effect. The most credited one is that EPA, present in large amounts in marine oils, may inhibit the expression of the gene of Δ^9 desaturase in the mammary gland, together with other mechanisms like the inhibition of mRNA of the lipase which acts on mammary lipoproteins. The experimental results cited by Chilliard *et al.*, (2001) give credit of an $R^2 = 0.75$ for the correlation between the amount of administered EPA and the intensity of depression of milk fat concentration. In particular, short and medium chain fatty acids (C_4 through C_{14}) are only slightly depressed, whereas PA, SA and OA are quite strongly depressed. The concentration of VA is remarkably enhanced, while that of the other trans 6, trans 9 and trans 10 isomers is much less increased. CLA as well are greatly augmented, passing from 0.2-0.6% of total lipids in control animals up to 1.5-2.7% in treated animals. The major CLA isomer is, as usual, RA (cis 9 trans 11), but also trans 10 cis 12 CLA is appreciably represented. It is necessary to be very careful to handle this particular point. As a matter of fact, the CLA which succeeded in escaping the rumen in excessive amounts could seriously jeopardise milk yield. Studies of Chouinard et al. (1999a, 1999b) clearly demonstrated that the infusion into the abomasum of 50-100 g/d of a commercial product based on a pool of CLA isomers results in a depression of more than 50% both of milk yield and milk fat. Such an effect is however to be attributed to the trans 10 cis 12 CLA isomer only, which commercial mixtures are rich in (Baumgard et al., 2000). The other isomers do not affect the amount of milk fat (Griinari et al., 1998; Chouinard et al., 1999b; Bauman & Griinari, 2001).

When fish oil is supplemented to the diets of beef cattle, n-3 PUFA are preferentially embodied within muscular phospholipids, both with unprotected and with formaldehyde protected oils (Ashes *et al.*, 1992), but usually adipose tissue depots, essentially made of triglycerides, are not affected. When n-3 PUFA are increased, usually AA is decreased. Increases of the same order of magnitude have been obtained with fish meal (Mandell *et al.*, 1997). Furthermore, the supplementation of the diets for beef cattle with marine oil results in increasing the amount of CLA and VA in intramuscular fat, exactly as it happens with milk (Enser *et al.*, 1999).

Vegetable fats

As far as vegetable fats are concerned, richer in unsaturated acids than animal fats, again they can be administered as either unprotected or rumen protected supplements. Experimental evidence clearly demonstrated that unprotected unsaturated acids exert a depression effect on milk yield and come up against more or less complete hydrogenation in the rumen. Unprotected fat is therefore to be avoided, unless it is still located inside the oilseed tissues. Thus, it is advisable that the use of vegetable fats in the diet of dairy cows be limited to rumen protected ones or to full fat oilseeds, adequately treated against anti-nutritional factors.

The recent paper by Chouinard et al. (2001) is particularly enlightening in this respect. Different energy supplements are compared with one another: calcium soap of canola oil; calcium soap of soybean oil; calcium soaps of linseed oil; full fat soybean seeds, extruded, micronised and toasted; marine oil. CLA had a three- to fivefold increase with calcium soaps, independently on the vegetable oil, with reference to control animal with no soaps in their diets. With full fat soybean CLA had a two- threefold increase and with marine oil about a threefold increase. It is concluded that the use of calcium soaps must be considered the best means to supplement energy to the diets of dairy cows in negative energy balance. It results in upgrading also the quality of milk in terms of CLA content, in a way comparable, in this respect, to the use of full fat soybean. The results obtained with fish oil were decidedly worse, also because a marked depression of milk yield occurs as well. The 2001 study of Chouinard and co-workers confirms a previous one (Chouinard et al., 1998a).

Solomon *et al.* (2000) too, essayed full fat extruded soybean seeds trying to put in evidence a possible associative effect with high amounts of non structural carbohydrates (starch and pectins). The associative effect was not detected, but the use of full fat soybean made milk yield increase of about 10%, with a decrease of short-medium chain fatty acids (C₈ through C₁₆), a decrease of SA and OA and the doubling of the concentration of CLA. The authors found a good correlation ($\mathbb{R}^2 = 0.77$) between the concentrations of VA and CLA, so confirming the validity of the previously expressed theories about the metabolic pathways relative to these acids in the rumen.

Bas and Morand-Fehr (2000), in a literature review of theirs, reported the effects of some nutritional factors on the acidic composition of the fat present inside the meat of lambs and emphasised that the depot fat stored prior to weaning exerts a remarkable influence upon the composition of both adipose and muscular tissues even several months after weaning. The effect should be similar to that exerted on the tissue lipids of monogastric animals (Kramer *et al.*, 1998; Park *et al.*, 1999). Bas and Morand-Fehr (2000) refer also that diets rich in beet pulps or fish meal favour OA to be increased in meat to the detriment of SA, LA and LNA,

Table 2

whereas the inclusion of maize or cotton meal in the ration increases the concentration of SA and LA in the depot fat. Such a modification of the saturated to unsaturated acids ratio obviously has an influence on the consistence of both the depot fat and the muscular fat. With goats, high levels of concentrate in the diet induce, on the contrary, an increase of branched and odd number acids in the depot fat. Furthermore, the level of SA in the adipose tissue decreases with increasing the weaning period (Banskalieva *et al.*, 2000).

Perez Alba *et al.* (1997) some years before were among the first researchers to study calcium salts of olive oil, as supplements of diets for dairy ewes. Their major results were: milk yield was increased, short and medium chain fatty acids (C_6 through C_{16}) and LA were decreased and SA and OA were increased. Unfortunately CLA was not yet so popular and didn't receive the attention it deserves.

Antongiovanni *et al.* (1999) compared calcium salts of olive oil to those of palm oil. Even confirming that the use of soaps may decrease the concentration of short and medium chain fatty acids, these Authors observed that with the use of calcium salts of olive oil the fat which is obtained is relatively richer in these acids (C_6 through C_{14}) than

Type of forage	Lowland pasture	High hill pasture	Herb silage	Maize silage
% ration DM	100	100	63	68
Fatty acids:				
C4:0	35	48	32	34
$C_{6:0} + C_{8:0}$	32	31	32	41
$C_{10:0} + C_{12:0}$	58	45	73	82
C14:0	99	96	125	124
C _{16:0}	258	249	356	327
C _{16:1}	17	18	16	22
C18:0	114	108	99	81
C _{18:1} trans 10 + trans 11	21	37	-	7
C _{18:1}	279	283	209	195
C18:2	26	45	15	22
C18:3	14	15	5	3
CLA	9	16	5	5

Effect of forego on the pointie composition (alle limite) of will fat

(modified from: Kelly et al., 1998; Chilliard et al., 2001; Collomb et al., 2001; Onetti et al., 2001)

the fat obtained with calcium salts of palm oil, whereas long chain acids (C_{16} through C_{22}) were less represented; n-3 acids were comparable.

With a trial carried out with Italian Friesian cows, Secchiari et al. (2003) compared 4 different feed lipid sources: calcium salts of olive oil; soybean meal coated with a protection layer of calcium salts of palm oil; full fat linseed and toasted full fat soybean. Since it is evident from the literature that soybean (either extruded or toasted) provides the best results in terms of quali- quantitative milk yield, the diet with full fat soybean was referred to as the control diet. The following results were achieved: calcium salts of olive oil promoted the highest milk yield, about 5% higher than all the other diets, yet with a lower fat level (3% vs. 3.5%). As far as fatty acids were concerned, full fat soybean was confirmed as the best energy supplementing feed with medium-short chain saturated acids at a lower level and C18 unsaturated acids at a higher level. Olive oil calcium salts exhibited an intermediate behaviour. The worst results were those with palm oil calcium salts. Lastly, the results referable to n-3 and CLA were quite interesting. Again full fat soybean proved itself as the best lipid source and palm oil the worst one, even though calcium salts of olive oil were comparable to those of palm oil in terms both of CLA and EPA. The only flaw, if we may say so, was that soybean produced the highest amount of trans 10 C_{18:1}. VA should be preferable because it doesn't result in fat depression.

In a similar way, also in the case of beef bullocks, the suppementation with oil seed rich in PUFA changes the acidic composition of both the adipose and the muscular tissues. As an example, the supplementation with rapeseed of a diet based on cereals and straw favours both the decrease of PA and the increase of all the C₁₈ acids (Geay et al., 2001). In this case too, a preferential distribution of some fatty acids within polar and neutral lipids occurred: intramuscular lipids contained higher amounts of OA and LA than those present inside the adipose depot. Furthermore, the supplementation with this lipid source led to an increase, in the depot fat, of vitamin E, an anti-oxidant factor, which preserves unsaturated acids from peroxidation. However, this effect results enhanced if supplementation is made with rumen protected fats. Linseed appears to be the most efficient lipid feed in increasing the level of α -LNA (Table 5).

The diet as a modifying factor of the level of CLA in fat produced by ruminants

The content of CLA in milk fat depends on the production of CLA itself and of VA in the rumen, as well as on the tissue metabolic activity of Δ^9 desaturase upon the precursors of ruminal origin. Hence dietary factors play an important part. Feeds for ruminants may be classified in categories according to how and how much they contribute to promote such beneficial compounds. The

Table 4.	Effect of the diet on the acidic composition of intramuscular
	fat of beef bullocks (g/100 g lipids).

Type of forage	Herb silage	Pasture	Нау
% ration DM	60	100	10
Fatty acids:			
C10:0	0.25	0.12	0.13
C12:0	0.09	0.09	0.08
C14:0	2.76	2.71	2.34
C14:1	0.63	0.66	0.60
C15:0	0.58	0.66	0.59
C 16:0	26.55	22.84	27.4
C16:1	3.73	3.88	3.98
C 17:0	1.20	1.20	1.22
C17:1	0.97	1.05	1.19
C 18:0	16.04	14.72	15.95
C18:1	39.47	40.58	38.64
C 18:2	2.60	2.11	2.96
CLA	0.47	1.08	0.37
C 18:3	0.71	1.13	0.72
C20:0	0.05	0.09	0.23
C _{20:1}	0.07	0.12	0.04
C20:2	0.09	0.34	0.07
C20:5	0.20	0.23	0.12
C20:3	0.14	0.38	0.09
C _{20:4}	0.14	0.32	0.10
n-6/n-3	3.91	2.33	4.15
PUFA/SFA	0.09	0.13	0.09
(man dified frame . F		<u></u>	

(modified from: French et al., 2000)

first category includes all those dietary factors which promote the production of CLA and VA in the rumen; the second category includes factors that modify the rumen environment by interfering with bacterial micro-flora; lastly, the third group comprises the feeds which, associated with each other, furnish a pool of lipids that modifies the bacterial population (Bauman et al., 1999). Feeds particularly rich in LA favour CLA synthesis because LA, when present in high amounts in the rumen environment, inhibits the reduction of VA, which in turn is desaturated in the mammary gland. In vivo trials showed that the response is dosedependent. On the contrary, in vitro experiments seem to clarify the reaction mechanism: it appears that LA acts as a competition inhibitor in the microbial bio-hydrogenation of VA to SA (Harfoot and Hazlewood, 1988) (Figure 4). The supplementation of diets with oil from seeds such as sunflower, soybean, maize, rapeseed, linseed, which usually affect the rumen microbial metabolism, should increase CLA in milk fat (Harfoot et al., 1973). Feeding dairy cows rations with linseed oil (4.4% on the DM basis) or soybean oil (4% on DM). an increase of CLA in milk fat over the control of

12.4 mg/g fat and 15.8 mg/g fat, respectively, could be observed. On the other hand, supplementing with 300g/d sunflower oil, CLA was increased by 0.3% and greater effects were obtained with the addition of a carbonate buffer or when the oil was in the form of calcium salts (Jahreis et al., 1999). But a much greater increase is achieved when the oil seed are treated with extrusion, micronisation or toasting processes (Chouinard et al., 1997; 2001; Secchiari et al., 2003). Unbroken full fat seeds do not alter the concentration of CLA because the fatty acids contained inside the seed body are not accessible to microbes which, hence, cannot operate any transformation. Feeding trials with dairy cows fed extruded soybean or cotton seeds (12% on the ration DM) showed that CLA can be increased from 3.4% of the control animals up to 6.9% and 6.0% with soybean and cotton, respectively (Dhiman et al., 1998).

The use of fish oil, rich in PUFA, in the rations for dairy cows increases the level of CLA in milk because of its inhibitory effect on rumen biohydrogenations, absolutely analogous to the effect of LA with the consequent accumulation of VA. Hence, it is to be assumed that, when fish oil is

In deer dulloc	ks (g/100	g lipias).				
Fat source	C 16:0	C _{18:0}	C18:1	C _{18:2}	C 18:3	CLA
Control (no fat supplement)	27.3	18.5	40.0	2.0	0.6	-
Animal fat:						
Tallow	28.2	19.6	37.5	1.8	0.4	-
Unprotected vegetable fats:						
Soybean	25.6	19.6	41.3	2.4	0.7	-
Rapeseed	24.3	21.2	40.6	2.0	0.7	-
Sunflower	24.4	19.5	42.4	2.7	1.1	-
Linseed	25.3	15.0	40.1	4.1	2.3	0.8
Cotton	28.0	18.5	39.0	2.3	0.6	-
Palm	30.6	18.2	37.8	2.5	1.2	-
Safflower	25.5	19.2	40.4	5.6	0.9	-
Protected vegatable fats:						
Rapeseed	22.7	19.3	39.6	9.1	1.1	-
Sunflower+soybean	21.8	21.3	34.2	11.8	1.0	-
Cotton+sunflower+safflower	22.6	20.7	37.8	13.7	0.6	-

Table 5. Effect of fat supplementation on the acidic composition of intramuscular fat in beef bullocks (q/100 q lipids).

(Modified from: Enser et al., 1999; Andrae et al., 2001; Bas and Sauvant, 2001)

the fat supplement, synthesised CLA comes for the greatest part from desaturation of VA in the mammary tissue (Chilliard et al., 1999). Variations of the concentration of VA may be also correlated to the fall of rumen pH (therefore to a modification of the microbial population) which occurs when the diet is characterised by a high level of concentrate and a low level of fibre; in fact, if a buffer is added and pH is maintained at normal levels, the synthesis of VA drops down (Griinari et al., 1998). Actually, it is not so easy to be able to distinguish the effect of the lowering of pH from the effect of the quality of the lipid substrate. As a matter of fact, in the literature contradictory examples of diets, unbalanced towards high levels of concentrate, inducing either an increase or a decrease of VA can be found (Chouinard et al., 1998a; Griinari et al., 1998). When diets with the same lipid composition were compared, it could be demonstrated that the fall of rumen pH does not induce any quantitative variation of trans octadecenoic acids, but inside this group of isomers, an important decrease of the synthesis of trans 11 in favour of trans 10 occurs and the latter becomes predominant (Griinari et al., 1998; 1999; Griinari, 2001). The amount of CLA increases remarkably when the cows are on pasture, as clearly demonstrated when diets based on pasture and based on hay with the same level of fat were compared (see above). The lipid of green herbage consist basically of glycolipids and phospholipids which are about 2% of DM. Some in vitro studies showed how glycolipids are hydrolysed and reduced by rumen microbes in a similar way as triglycerides (Kelly et al., 1998; Bauman et al., 1999). Also the stage of maturity of the herbage appears to influence the concentration of CLA: with young forage crops the amount of CLA in milk fat is increased with respect to milk fat from cows fed more mature herbage or later cuts (Chouinard, 1998b). Besides, the level of CLA is higher in Spring and Autumn and lower in the other seasons. However, the quali- quantitative composition of the fat fraction of green herbage appears not completely able to explain the influence exerted over the CLA level in milk. Most probably some synergic effects exist between the lipid substrate and other components present in the feed which may inter-react with rumen fermentation.

The supplementation with fish oil results in increasing the aliquot of trans C_{18:1}, especially of trans 11 with the consequent enhancement of RA and of trans 10. According to some Authors, this latter acid would depress milk fat synthesis (Bauman and Griinari, 2001; Chilliard et al., 2001). One more factor which may promote variations are ionophors, which inhibit the growth of gram-positive bacteria involved in bio-hydrogenation such as Butyrivibrio fibrisolvens. Supplementing the diets of dairy cows with ionophors could inhibit the complete hydrogenation of LA, so favouring the accumulation of VA (Figure 4). But results from the literature are contradictory on this subject. The most likely hypothesis is that such behaviour be linked to the capability of ionophor-resistant bacteria to replace the sensitive ones (Bauman et al., 1999).

Studies with lactating cows demonstrated that the level of CLA in milk fat is proportional to its level of diet supplementation. Diets supplemented with CLA mixtures, mainly cis, *trans* 8/10; 9/11; 10/12 and 11/13, put in evidence the remarkable efficiency of transfer of the whole pool of CLA isomers to milk (Chouinard *et al.*, 1999a; 1999b). Similarly to PUFA, also in the case of CLA the most difficult barrier to be passed is the rumen wall: by means of abomasal infusions of CLA mixtures, about 50% was transferred to milk fat (Chin *et al.*, 1992). Moreover, a very good transfer efficiency of dietary CLA into milk fat was achieved by protecting from possible hydrogenation the CLA mixture inside a protein matrix capsule (Gulati et al., 2000).

Conclusions

Conclusions may be simply listed as the following points:

- it is possible to modify the quality of milk and meat fat by dietary means;
- the forage basis is a determining factor with respect to the quality of milk and meat fat;
- grazed forage gives the best results, especially in the case of high hill pastures: short chain fatty acids (< C₁₀) are increased; medium-long chain fatty acids (C₁₂ - C₁₆) are decreased; n-3 and CLA are increased;

- the addition of fats or fatty feeds to supplement energy is the only option if pasture or fresh forage are not available;
- fats of animal origin are to be avoided, fats and feed of plant origin are to be preferred;
- in order to avoid as much as possible the natural bio-hydrogenation processes in the rumen, fats must be protected. The best protection is their saponification to calcium salts. As an alternative, full fat oil seed may be used, provided they are adequately treated (extrusion, toasting, etc.), the integument and cell wall of which being a sufficient protection of the inside lipids;
- the effects of supplementation either with full fat seeds or with calcium soaps on milk and meat fat are exerted on: yield, which may be enhanced, even though not always; medium-long chain fatty acids (C₁₂ C₁₆), which decrease; SA and OA, which increase; n-3 and CLA, which increase as well;
- among the calcium soaps, those of olive oil turned out to be the best ones, followed by those of palm oil;
- among the full fat seeds, toasted soybean showed itself as the most efficacious, superior to calcium salts, even though *trans* 10 $C_{18:1}$, responsible for milk fat depression (MFD), appeared to be increased along with the other C_{18} acids.

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