Part IV

Appendix

23

Improving the nutritional quality of milk

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23.1 Introduction

Milk is a food of outstanding interest, not least because it was designed to be a complete food for young growing animals. The role of bovine milk in the human diet as a source of fat, amino acids and other nutrients, including calcium, has been recognised for centuries. Milk is a complex colloid consisting of globules of milk fat suspended in an aqueous solution comprised of lactose, proteins (primarily caseins), mineral salts and water-soluble vitamins. Typical milk from Holstein/Friesians contains about 40, 36 and 45 g/kg of fat, protein and lactose respectively, with an energy content of 2.8 MJ/kg. However, these values vary considerably between different breeds and in response to variations in nutrient supply.

Milk proteins are of high nutritional quality with a biological value and net protein utilisation approaching 100%. As a result, recent research has been directed towards maintaining or enhancing milk protein content and examining factors that control the secretion of casein proteins that are of particular importance with respect to cheese making. In addition, there has been considerable interest in the relationship between milk fat consumption and human health.

There is now a general consensus that the consumption of foods rich in saturated fatty acids (SFA) is associated with an increased risk of cardiovascular disease and the development of insulin resistance and dyslipidaemia (Vessby *et al.*, 2001). Higher intakes of *trans* fatty acids are also thought to be associated with increased cardiovascular risk (Willet *et al.*, 1993), whilst more recent studies have clearly demonstrated the important and positive role of *cis*-monounsaturates (*cis*-MUFA) and long chain n-3 polyunsaturates (n-3 PUFA) in the human diet.

Notably, a recent comprehensive study across 14 Western European countries (Hulsof *et al.*, 1999) identified that milk and dairy products contribute up to 40, 58 and 72% of total fat, SFA and *trans* fatty acid intake, although these values vary considerably between individual countries. However, it is important to recognise that the functionality of *trans* fatty acids from milk and dairy products remains unclear, and it is possible that some may be beneficial. In view of the potential benefits, there has been intense interest in manipulating milk fatty acid composition with the overall aim of improving the long-term health of the population. One of the major strategies has been to simultaneously reduce the proportion of $C_{12:0}$ to $C_{16:0}$ and increase those of MUFA and n-3 PUFA.

Lactose is a disaccharide normally readily digestible, but some individuals lose the ability to produce intestinal lactase as children or later in life and develop lactose intolerance, a condition that causes bloat, pain and diarrhoea. The incidence of lactose intolerance is particularly high in non-white races. Manipulation of milk lactose content is extremely limited and is therefore not considered in more detail.

Milk composition can be manipulated by nutritional means or utilising natural genetic variation. Genetic improvement can be attained through exploitation of between- (cross-breeding) or within- (selection) breed variation, while recent advances in genetic engineering offer the promise of changes not previously possible using traditional nutritional and genetic approaches (Karatzas and Turner, 1997). Nutrition can be used to manipulate milk fat content and composition, but concentrations of protein are much less responsive (Sutton, 1989), and therefore genetic selection represents a more effective long-term strategy for enhancing milk protein content.

23.2 Factors affecting milk protein content

Protein is the most valuable milk constituent and is influenced by nutritional, physiological and genetic factors (Erasmus *et al.*, 2001). Milk protein constitutes about 95% of total milk nitrogen, and is comprised of caseins (α, β, κ and γ), whey proteins (β-lactoglobulin and α-lactalbumin), serum albumin and immunoglobulins. Even though whey proteins are of high nutritional value, only the casein fraction is important to cheese makers. Casein accounts for between 76 and 86% of total milk protein (DePeters and Cant, 1992) and is essentially independent of nutrition and stage of lactation (Coulon *et al.*, 1998). Non-protein nitrogen fractions (urea, peptides, amino acids, creatinine and purine metabolites) appear in milk as a result of diffusion from the peripheral circulation, while milk proteins, other than serum albumin and immunoglobulins, are synthesised in mammary epithelial cells from amino acid precursors.

Milk protein content is dependent on both breed and stage of lactation, as well as nutrition. Breeds that produce milk with a high fat content also have higher protein concentrations, but the ratio of protein to fat is lower for Channel

Island breeds compared with the Ayrshire, Holstein or Friesian. In addition to changes during lactation, milk protein content is also affected by parity and progressively decreases in animals above three years old, such that across five successive lactations a 4% reduction can be expected (Erasmus *et al*., 2001). Changes in protein content across lactations are also associated with a decline in the proportion of casein in total milk protein, but there is little consensus on the extent of decrease that can be expected.

23.2.1 Effects of energy intake

Nutrition also affects milk protein content. It is well established that energy intake is the most important dietary attribute influencing milk protein content. Across a wide range of diets, an increase in one MJ of metabolisable energy (ME) intake has been estimated to stimulate 0.2–0.3% increases in milk protein content (Spörndly, 1989). Coulon and Remond (1991) estimated that in early and mid-lactation cows an additional MJ of ME intake could be expected to result in respective 0.04 and 0.08 g/kg increases in milk protein content. However, these changes do not appear to be accompanied by improvements in the proportion of casein in total milk protein (Coulon *et al*., 1998).

While the relationship between energy intake and milk protein synthesis is widely accepted, it is clear that this holds true only when increases in energy are derived from carbohydrates and protein. Feeding fat supplements generally results in a 1–4 g/kg depression in milk protein content (Spörndly, 1989; Sutton, 1989; DePeters and Cant, 1992). In practice, increases in energy intake can be achieved with concentrate supplements or replacing grass silage with forages of higher intake potential and/or energy content. Increases in concentrate supplementation generally increase milk protein content but responses are unpredictable and vary between -0.06 to 0.82 g/kg per kg concentrate DM intake (Shingfield, unpublished data).

23.2.2 Effects of type of forage

The intake and milk production potential of maize silage is higher than grass silage (e.g. Fitzgerald and Murphy, 1999). Replacing grass with maize silage consistently increases milk protein content, even when maize silage contains relatively low amounts of starch (Phipps *et al*., 2000). It is a popular perception that these improvements are related to higher intakes of energy and the amount of starch available for absorption from the small intestine but this explanation ignores the changes in rumen fermentation and post-absorptive metabolic and endocrine status (Reynolds *et al*., 1997). Furthermore, post-ruminal infusions of maize or wheat starch have not stimulated appreciable increases in milk protein content (Reynolds *et al*., 2001). Feeding maize silage would be expected to increase the amount of amino acids available for absorption, since microbial protein synthesis is essentially a function of organic matter intake (Shingfield, 2000) and is energetically more efficient for maize than grass silage based diets

(Givens and Rulquin, 2002), and ruminal propionate production is also enhanced (Fitzgerald and Murphy, 1999). It appears that at least part of the increase in milk protein content associated with feeding maize silage may be explained by improvements in amino acid and gluconeogenic precursor supply.

23.2.3 Amino acid supply

Numerous studies have shown that arterial essential amino acid concentrations and milk protein synthesis increase in response to abomasal infusions of casein (e.g. Hanigan *et al.*, 2001) leading to the general conclusion that the amino acid composition of protein entering the duodenum is the single most important nutritional factor influencing milk protein content (Erasmus *et al.*, 2001). Enhancing arterial amino acid concentrations underpins nutritional strategies for enhancing milk protein content by promoting increases in amino acid availability to the mammary gland and/or improving the supply of essential amino acid that limits milk protein synthesis. As a result there has been considerable interest in replacing protein supplements of relatively high degradability with less degradable sources to increase the flow of amino acids entering the duodenum. Often, the improvements in milk protein yield are realised through increases in milk yield, rather than milk protein content.

Research has been directed towards determining the relative deficiencies of individual amino acids in order to identify the most appropriate protein supplements for various diets. Post-ruminal infusions have implicated lysine and methionine as first and second limiting for milk and milk protein synthesis in most diets. In cases where maize and maize by-products provide the majority of rumen undegraded protein, lysine appears to be first limiting, but for maize based diets supplemented with animal or vegetable proteins, methionine is thought to be first limiting (Polan *et al.*, 1991, Schwab *et al.*, 1992). For grass silage based diets histidine (Kim *et al.*, 1999; Vanhatalo *et al.*, 1999), rather than leucine (Huhtanen *et al.*, 2002), methionine or lysine (Varvikko *et al.*, 1999) appears first limiting. Even when post-ruminal infusions of single amino acids have stimulated increases in milk protein, responses are generally confined to an increase in yield rather than concentration.

23.3 Factors affecting milk fat content

In contrast to milk protein, nutrition can be used to effect substantial changes in milk fat content and milk fatty acid composition.

23.3.1 Milk fat synthesis

Milk fat is comprised of a complex mixture of lipids, most of which are present as triacylglycerides (about 98%), in addition to small amounts of di- and monoacylglycerides, phospholipids, cholesterol and non-esterified fatty acids

(Christie, 1995). Fatty acids secreted in milk originate from two sources, direct incorporation from the peripheral circulation and *de novo* synthesis in the mammary gland. *De novo* synthesis accounts for all $C_{4:0}$ to $C_{12:0}$, most of the $C_{14:0}$ and about half of $C_{16:0}$ secreted in milk, while all C_{18} and longer chained fatty acids are derived entirely from circulating blood lipids (Hawke and Taylor, 1995). A distinctive feature of the bovine mammary gland is the ability to release fatty acids from the synthetase complex at various stages, resulting in the secretion of a wide range of short and medium chain fatty acids.

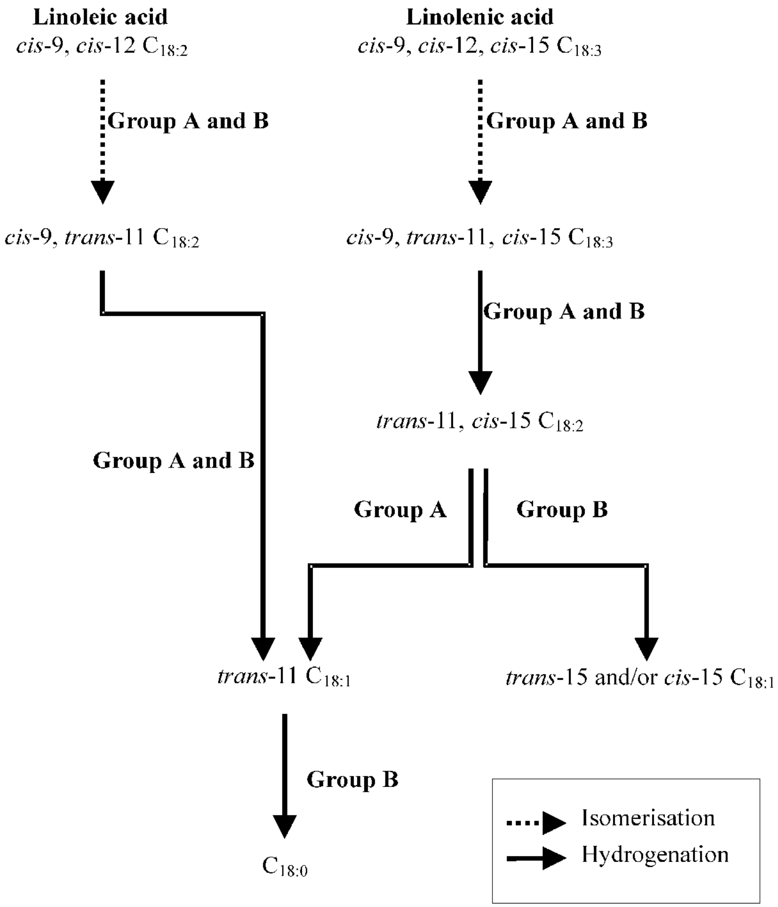
Due to extensive metabolism of dietary unsaturated fatty acids in the rumen, $C_{18:0}$ is, under normal conditions, the predominant long chain fatty acid available for absorption. However, *cis*-9 $C_{18:1}$ output in milk exceeds uptake due to the activity of stearoyl Co A (Δ-9) desaturase in mammary secretory cells (Kinsella, 1972). The introduction of the *cis*-9 double bond is thought to occur as a means of ensuring milk fluidity necessary for efficient ejection from the mammary gland (Grummer, 1991). Conversion of $C_{18:0}$ to *cis*-9 $C_{18:1}$ is the predominant precursor:product of the Δ-9 desaturase, and about 40% of $C_{18:0}$ taken up by the gland is desaturated (Chilliard *et al.*, 2000). In addition to $C_{18:0}$, both $C_{14:0}$ and $C_{16:0}$ are also converted, whilst more recent studies have shown that *trans*-11 $C_{18:1}$ (Griinari *et al.*, 2000) and *trans*-7 $C_{18:1}$ (Corl *et al.*, 2002; Piperova *et al.*, 2002) are desaturated to *cis*-9, *trans*-11 $C_{18:2}$ and *trans*-7, *cis*-9 $C_{18:2}$, respectively.

23.3.2 Lipid metabolism in the rumen

It is well established that ruminant lipids contain much higher levels of SFA relative to ingested dietary lipids as a result of extensive biohydrogenation in the rumen. Data from both *in vitro* and *in vivo* studies have allowed the major biohydrogenation pathways to be elucidated (Harfoot and Hazlewood, 1988; Fig. 23.1). Metabolism of $C_{18:3}$ (n-3) proceeds via isomerisation to a $C_{18:3}$ conjugate (*cis*-9, *trans*-11, *cis*-15), followed by sequential reduction of double bonds to *trans*-11, *cis*-15 $C_{18:2}$, *trans*-11 $C_{18:1}$ (trans vaccenic acid, TVA) and $C_{18:0}$. Biohydrogenation of $C_{18:2}$ (n-6) involves an initial isomerisation to *cis*-9, *trans*-11 $C_{18:2}$ (conjugated linoleic acid, CLA) and successive reduction to form *trans*-11 $C_{18:1}$ and $C_{18:0}$. The final reduction is considered to be rate limiting, such that *trans* $C_{18:1}$ can accumulate in the rumen (Griinari and Bauman, 1999). Bacteria that convert *trans* $C_{18:1}$ fatty acids to $C_{18:0}$ are also thought to metabolise $C_{18:1}$ (n-9). Biohydrogenation of unsaturated fatty acids is extensive, and under normal conditions about 80% (range 70–95) of $C_{18:2}$ (n-6) and 92% (range 85–100%) of $C_{18:3}$ (n-3) are metabolised in the rumen (Doreau and Ferlay, 1994).

23.3.3 Altering milk fat content

Breed has a marked effect on milk fat, and concentrations are between 19 and 30% higher for Jerseys than Holstein dairy cows (Drackley *et al.*, 2001; White *et al.*,



Note: Group A and B refer to different rumen bacteria capable of biohydrogenation characterised according to Kemp and Lander (1984).

Fig. 23.1 Major metabolic pathways of ruminal biohydrogenation of polyunsaturated fatty acids (adapted from Harfoot and Hazlewood, 1988).

2001). Milk fat content is also dependent on the stage of lactation, and changes in much the same manner as milk protein content. Nutrition represents the most effective means of manipulating milk fat content (e.g. Palmquist *et al.*, 1993). Under normal conditions, increases in concentrate supplements have little impact on milk fat content (Table 23.1), until the proportion of forage decreases to below 50% of dietary dry matter (Thomas and Martin, 1988) or concentrates contain relatively high levels of polyunsaturated fatty acids (Griinari *et al.*, 1998). Decreases in milk fat content typically occur within a few days after changes in the diet and can occur in the absence of alterations in the secretion of milk or other milk constituents. During milk fat depression, secretion of all fatty acids is reduced, the extent of which is greater for those synthesised *de novo* (Bauman and Griinari, 2001).

Table 23.1 Typical milk production responses to increases in concentrate supplementation

Basal forage	Range in supplement offered (kg/day)	Mean response per kg concentrate DM intake[a]					Reference
		Milk yield (kg/day)	Milk protein content (g/kg)	Milk protein output (g/day)	Milk fat content (g/kg)	Milk fat output (g/day)	
Grass silage	3–6	1.6	0.30	61.0	0.23	70.3	Sutton *et al.* (1994)
Grass silage	2–4	0.61	0.50	25.5	0.39	31.1	Agnew *et al.* (1996)
Grass silage	4–6	1.12	0.35	37.7	−0.71	29.4	
Grass silage	6–8	0.59	0.82	34.3	−1.00	0.63	
Grass silage	2–8	0.77	0.56	32.4	−0.42	20.6	
Grass silage	2–4	1.93	−0.06	55.2	1.32	94.3	Keady and Murphy (1997)
Grass silage	4–6	1.11	0.58	38.2	0.46	46.8	
Grass silage	2–6	1.52	0.26	46.7	0.89	70.6	
Grass silage	4–6	0.82	0.12	29.4	0.06	35.3	Fitzgerald and Murphy (1999)
Grass silage	6–8	0.95	0.42	36.8	−0.53	21.1	
Grass silage	4–8	0.89	0.28	33.3	−0.25	27.8	
Maize silage	4–6	0.41	0.65	29.4	−0.24	17.7	Fitzgerald and Murphy (1999)
Maize silage	6–8	0.47	0.35	23.5	0.94	47.1	
Maize silage	4–8	0.44	0.50	26.5	0.35	32.4	

[a] Responses calculated as the difference between treatment controls and supplemented diets and expressing the response on a kg concentrate DM intake basis.

Feeding high starch concentrate supplements are generally thought to be associated with milk fat depression (e.g. Keady *et al*., 1999), but replacing starch with fibre has in some cases also decreased milk fat content (e.g. Huhtanen *et al*., 1995). Even though the impact of changes in concentrate energy source on milk fat are often unpredictable, switching cereals for soluble carbohydrates, and replacing starch for digestible fibre has been suggested as an effective strategy for alleviating milk fat depression on low forage diets (Sutton, 1989).

Lipid supplements are generally used to increase dietary energy content, but have variable effects on milk fat content that are dependent on inclusion rate, degree of unsaturation and physical form (Sutton, 1989). Fish oil consistently depresses milk fat content, irrespective of the basal diet (Table 23.2). A number of theories have been proposed to explain dietary induced milk fat depression, that attribute the lowered milk fat secretion to (i) reductions in acetate and butyrate production in the rumen limiting mammary *de novo* fatty acid synthesis, (ii) increased production of propionate and glucose causing increased insulin secretion resulting in absorbed fatty acids being partitioned towards adipose at the expense of the mammary gland or (iii) direct inhibition by *trans* fatty acids produced during ruminal biohydrogenation of dietary unsaturated fatty acids. None of these theories offer a universal explanation for this phenomenon, but the *trans* fatty acid hypothesis is currently thought to be the most robust (Bauman and Griinari, 2001). Recent work has established that post-ruminal infusions of *trans*-10, *cis*-12 CLA, but not *cis*-9, *trans*-11 CLA, inhibit milk fat synthesis (Fig. 23.2), in a dose dependent manner (e.g. Baumgard *et al*., 2000; Peterson *et al*., 2002).

23.3.4 Altering milk fatty acid composition

In addition to the effects on milk fat content, milk fat concentrations of $C_{16:1}$, $C_{18:1}$ and CLA are higher, and that of $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$ and $C_{14:0}$ are lower for Holstein than Jersey dairy cows (White *et al*., 2001). Higher levels of $C_{16:1}$, $C_{18:1}$ and CLA are consistent with the view that the activity of Δ^9-desaturase is higher in Holstein mammary tissue (Beaulieu and Palmquist, 1995), while the higher concentrations of short and medium chain fatty acids suggest a greater proportion of Jersey milk fat is synthesised *de novo*. Stage of lactation also affects the proportion of fatty acids derived from *de novo* synthesis with concentrations of short and medium chain fatty acids being lower in early lactation.

Use of dietary lipid supplements is the most common nutritional means for manipulating milk fatty acid composition. However, both the type and source of dietary fat affect the magnitude of changes that can be achieved (Table 23.3). Supplements of plant oils or oilseeds reduce short and medium chain but increase long-chain fatty acids in milk, resulting in an overall shift towards $C_{18:0}$ at the expense of $C_{16:0}$ (Mansbridge and Blake, 1997) due to decreased *de novo* synthesis and/or reduced mammary uptake of absorbed $C_{16:0}$. Reductions in milk unsaturated fatty acid content are characterised by small increases in the

Table 23.2 Typical milk production responses to dietary lipid supplements

Lipid	Basal forage	Inclusion (g/kg) DM)	Mean response[a]					Reference
			Milk yield (kg/day)	Milk protein content (g/kg)	output (g/day)	Milk fat content (g/kg)	output (g/day	
Fish oil	Grass silage	16	0.4	−3.0	−35	−10.9	−167	Offer *et al*. (1999)
Fish oil	Grass silage	31	3.2	−3.8	7	−15.0	−250	Keady *et al*. (2000)
Fish oil	Grass/maize silage	37	−4.5	−2.4	−198	−10.0	−358	Ahnadi *et al*. (2002)
Fish oil	Maize silage	17	1.5	−0.9	16	−13.3	−300	Chilliard and Doreau (1997)
Linseed oil	Grass silage	16	1.8	−1.1	41	−0.8	69	Offer *et al*. (1999)
Rapeseed oil	Maize silage	35	−1.9	−1.7	−112	0.0	−56	Jenkins (1998)
Rapeseed oil	Maize silage	33	0.8	−0.7	3	−2.8	−80	Loor *et al*. (2002)
Soyabean oil	Maize silage	35	0.4	−0.1	10	−6.7	−189	Jenkins *et al*. (1996)
Sunflower oil	Maize silage	37	−0.8	−1.2	−59	−3.0	−104	Kalscheur *et al*. (1997)
Tallow	Maize silage	40	−4.2	0.9	−101	−3.0	−253	Onetti *et al*. (2001)

[a] Responses calculated as the difference between treatment controls and lipid supplemented diets.

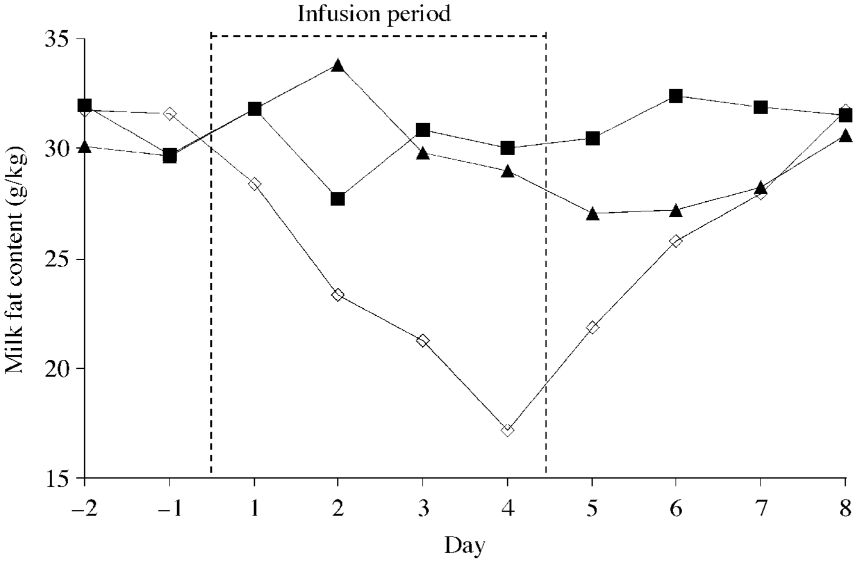


Fig. 23.2 Effect of abomasal infusions (10g/day) of control (), *cis*-9, *trans*-11 CLA (_) and *trans*-10, *cis*-12 CLA (—) on milk fat content (data derived from Baumgard *et al.*, 2000).

concentration of the predominant fatty acid in lipid supplements. In all cases, feeding plant oils increases milk fat $C_{18:0}$, *cis*-9 $C_{18:1}$ and *trans* $C_{18:1}$ content due to extensive ruminal metabolism of long chain fatty acids, leading to an increase in the supply of biohydrogenation intermediates and $C_{18:0}$ to the mammary gland.

It is notable that PUFA are not synthesised in any appreciable quantities in ruminant tissues and therefore concentrations in milk are primarily dependent on the amount leaving the rumen. As a result, oils rich in $C_{18:2}$ n-6 and $C_{18:3}$ n-3 have been used with the aim of increasing the concentrations of these PUFA in milk fat. In addition, much work has been carried out with the aim of increasing the concentrations of the long chain n-3 PUFA eicosapentaenoic (EPA, $C_{20:5}$ n-3) and docosahexaenoic (DHA, $C_{22:6}$ n-3) acids in milk. This has normally involved feeding fish oil supplements rich in EPA and DHA. However, the transfer efficiency into milk of EPA and DHA is low due to extensive biohydrogenation in the rumen and transportation in phospholipid and cholesterol ester fractions of plasma that are poorly utilised by the mammary gland (Rymer *et al.*, 2003).

A number of technologies have been developed to protect lipids from ruminal metabolism to reduce biohydrogenation and minimise the negative effects of dietary fat on animal performance. The approaches used include encapsulating oils and oilseeds within a formaldehyde casein complex, producing calcium salts of non-esterified fatty acids or generating fatty acyl amides. Even though these products are often only partially protected from ruminal metabolism, they are

Table 23.3 Typical milk fatty acid responses to dietary lipid supplements

Lipid	Mean response[a]										Reference
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	*cis*-9 $C_{18:1}$	*trans* $C_{18:1}$	$C_{18:2}$	$C_{18:3}$	SAT	MUFA	PUFA	
Rapeseed oil	−0.03	−0.20	0.55	0.39	0.30	−0.09	0.11	−0.07	0.30	−0.04	DePeters *et al*. (2001)
Rapeseed oil	−0.19	−0.33	0.51	0.67	2.00	−0.04	0.60	−0.18	0.60	0.15	Loor *et al*. (2002)
Soyabean oil	−0.29	−0.28	0.45	0.23	4.61	0.33	–	−0.19	0.53	0.33	Jenkins *et al*. (1996)
Sunflower oil[b]	−0.32	−0.32	0.14	0.27	3.07	−0.05	−0.24	−0.22	0.53	0.12	Kalscheur *et al*. (1997)
Sunflower oil	−0.23	−0.26	0.15	0.11	2.79	0.07	−0.21	−0.17	0.36	0.21	Kalscheur *et al*. (1997)
Linseed oil	−0.11	−0.15	0.27	0.26	0.94	−0.14	0.17	−0.07	0.23	−0.06	Offer *et al*. (1999)
Fish oil	0.04	−0.01	−0.45	−0.25	8.08	0.24	0.03	−0.09	0.24	0.29	Offer *et al*. (1999)
Fish oil	0.30	0.35	−0.77	−0.73	2.19	0.39	0.07	−0.05	−0.10	2.17	Shingfield *et al*., in press
Tallow	–	0.06	0.04	0.22	0.19	−0.37	−0.37	−0.06	0.19	−0.36	Onetti *et al*. (2001)
Protected lipids											
Butylsoyamide[c]	−0.06	−0.05	0.04	−0.04	0.61	0.74	–	−0.04	0.01	0.74	Jenkins *et al*. (1996)
Canolamide[d]	−0.24	−0.33	0.76	0.74	0.93	0.04	0.40	−0.16	0.55	0.08	Loor *et al*. (2002)
Ca-salts of rapeseed oil	−0.24	−0.37	0.31	0.76	–	0.10	−0.20	−0.23	0.69	0.07	Chouinard *et al*. (1998)
Ca-salts of soyabean oil	−0.31	−0.37	0.35	0.73	–	0.23	−0.20	−0.23	0.63	0.19	Chouinard *et al*. (1998)
Ca-salts of linseed oil	−0.26	−0.37	0.34	0.54	–	0.36	0.40	−0.21	0.46	0.36	Chouinard *et al*. (1998)
Fish oil[e]	–	0.00	−0.36	−0.29	2.34	0.33	0.19	−0.10	0.19	0.45	Lacasse *et al*. (2002)
Soyabean/fish oil[fg]	−0.04	−0.27	−0.73	−0.29	2.34	1.39	1.00	−0.29	0.14	1.78	Gulati *et al*. (2003)
Soyabean/fish oil fh	−0.03	−0.25	−0.79	−0.44	3.15	0.69	0.33	−0.31	0.18	1.45	Gulati *et al*. (2003)
Rapeseed/soyabean oil[f]	−0.26	−0.35	−0.13	0.51	−0.14	5.50	2.00	−0.25	0.44	4.21	Ashes *et al*. (2000)

[a] Responses calculated as proportionate differences between treatment controls and lipid supplemented diets.
[b] Sunflower oil rich in *cis*-9 $C_{18:1}$.
[c] Prepared by reacting soyabean oil with butylamine
[d] Prepared by reacting rapeseed oil with ethanolamide.
[e] Encapsulated fish oil in a glutaraldehyde-treated protein matrix.
[f] Lipids encapsulated in a matrix of rumen-inert protein.
[g] Fish oil rich in $C_{22:6}$ (n-3).
[h] Fish oil rich in $C_{20:5}$ (n-3).

more effective in changing milk fatty acid composition compared with parent oils (Table 23.3). The apparent transfer of $C_{18:2}$ n-6 and $C_{18:3}$ n-3 have been increased from less than 0.05 to 0.35 and 0.42, respectively, in response to formaldehyde protected lipids (Grummer, 1991), but these changes can be too extreme, and result in a dramatic increase in the incidence of spontaneous oxidation and off-flavours.

23.3.5 Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a generic term used to describe a mixture of geometric and positional isomers of $C_{18:2}$ in which the double bonds are conjugated rather than methylene interrupted . There is now increasing evidence that CLA exhibits anti-carcinogenic properties and may also improve immune function (see review of Williams, 2000). Dairy products are the major source of CLA in the human diet, primarily as the c9, t11 isomer (Lawson *et al*., 2001) that is also responsible for the anti-carcinogenic effects in animal models. In view of these benefits, there has been considerable interest in understanding the mechanism that controls CLA synthesis in the dairy cow, with the overall aim of producing CLA enriched milk.

CLA is formed as an intermediate of $C_{18:2}$, c9, c12 biohydrogenation in the rumen that is subsequently reduced to *trans*-11 $C_{18:1}$ (TVA), an intermediate also generated during the biohydrogenation of $C_{18:3}$ n-3 (Fig. 23.1) Since the final reduction of *trans* $C_{18:1}$ is normally the rate limiting step in the biohydrogenation of unsaturated C18 fatty acids, *trans* $C_{18:1}$ can accumulate and leave the rumen. The ratio of CLA to *trans* $C_{18:1}$ in rumen fluid of around 1:40 (Griinari and Bauman, 1999) is much higher than milk fat (1:3) leading to the suggestion that a large proportion of CLA secreted in milk is produced by the mammary gland. Furthermore, diets rich in C18:3 (n-3) but low in C18:2 (n-6) have substantially increased milk fat *cis*-9, *trans*-11 CLA concentrations, even though this isomer is not produced during ruminal metabolism of C18:3 (n-3). Further studies have established that *cis*-9, *trans*-11 CLA is formed from TVA via Δ-9 desaturase in the mammary gland (Griinari *et al*., 2000) and endogenous conversion can account for up to 90% of the CLA in milk. Endogenous conversion also occurs in human tissues (Adlof *et al*., 2000) and therefore the occurrence of TVA in milk fat may also be beneficial.

23.4 Future trends

The overall nutritional value of milk as a food is not in doubt but there are concerns regarding the intake of certain fatty acids derived from milk and dairy products. At the same time there is considerable interest in fatty acids and lipids unique to milk fat including CLA, branched chain fatty acids and sphingolipids that may afford very considerable benefits in disease prevention. These may well explain the positive effects of milk fat consumption in several long-term epidemiological studies. Both milk fat content and fatty acid composition can be modified by various means, nutritional being the most effective. Even in the absence of more definitive data, certain positive changes could be implemented at the present time. However, for this to happen there must be a market driver to provide producers a financial incentive to deliver modified milk. Major progress in this area is unlikely until the dairy industry embraces these potential opportunities.

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