



## Improving the Quality of Products from Grassland

Nigel D. Scollan

*Institute of Grassland and Environmental Research, UK*

R. J. Dewhurst

*Institute of Grassland and Environmental Research, UK*

A. P. Moloney

*Teagasc, Ireland*

J. J. Murphy

*Teagasc, Ireland*

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The XX International Grassland Congress took place in Ireland and the UK in June-July 2005.

The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

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## Improving the quality of products from grassland

N.D. Scollan<sup>1</sup>, R.J. Dewhurst<sup>1</sup>, A.P. Moloney<sup>2</sup> and J.J. Murphy<sup>3</sup>

<sup>1</sup>*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Wales, SY23 3EB, United Kingdom*

*Email: nigel.scollan@bbsrc.ac.uk*

<sup>2</sup>*Teagasc, Grange Research Centre, Dunsany, Co. Meath, Ireland*

<sup>3</sup>*Teagasc, Moorepark Research Centre, Fermoy, Co. Cork, Ireland*

### Key points

1. Consumers are increasingly aware of the links between diet and health, and place increasing emphasis on nutritional quality as a component of product quality.
2. Meat and milk products are rich sources of nutrients such as omega-3 (*n*-3) fatty acids and conjugated linoleic acid, which offer health benefits to consumers.
3. Green plants are the primary source of *n*-3 fatty acids in the food chain.
4. Grassland production systems have the potential to enhance the content of beneficial fatty acids, improve stability (from higher antioxidant content) and alter sensory attributes of meat and milk.
5. Grassland offers considerable scope to help create product differentiation in increasingly competitive markets.

**Keywords:** consumer, health, grass, fatty acids, quality

### Introduction

The impetus to enhance the quality of animal products has increased in recent years as industry seeks to meet the rapidly changing requirements of consumers who require food that is safe, healthy, traceable, of consistent eating quality, diverse and convenient. In addition to consumer related issues, increased globalisation, reduced commodity prices, reform of Common Agricultural Policy in Europe, increased interest on animal welfare and environmental issues, have combined to reduce profit margins, particularly for the primary producer. Improving the quality of milk and meat is considered to maintain or increase consumption and help to *add-value* across the food chain.

Consumers are increasingly aware of the relationships between diet and health, particularly in relation to cancer and atherosclerosis. Knowledge of these relationships has increased interest in identifying food components that may improve health, and hence “nutritional” quality is becoming a more important dimension of product quality. Consumers often have a negative perception of food products derived from animals relative to plants. However, there is increasing evidence that the consumption of milk and meat and associated products may confer additional health benefits, including protection against cancer. Table 1 presents an overview of components in milk and meat that are considered to provide health benefits in relation to factors regulating their concentration. The primary factors relate to the crop and animal but it must be recognised that further modification may occur during manufacturing of the raw material or by fortification, for example addition of vitamins during processing to increase nutritional value. It is evident that the main components influenced by crop genetics and management are the lipids. This paper will focus on recent research on manipulating the fatty acid composition of milk and meat (mostly beef) and the implications for important quality characteristics such as colour shelf life and sensory attributes.

**Table 1** Factors influencing milk and meat components shown to have health benefits for man

	Crop		Animal			Manufac- turing	Fortifi- cation
	genetics	management	feeding	genetics	management		
Protein			✓	✓			
Bioactive peptides			(✓)	(✓)		✓	
Sphingolipids							
Stanols/sterols							✓
Ca	(?)		✓	✓			
Vitamins							
A			✓				✓
B <sub>6</sub> , B <sub>12</sub>						✓	✓
C							✓
D			✓				✓
E	✓	✓	✓	species			✓
Immunoglobulins					✓	✓	
Melatonin					✓		
Fatty acids							
<i>n</i> -3 PUFA	✓	✓	✓			✓	✓
CLA/TVA	✓	✓	✓	✓		✓	
Iso-C15:0			✓			✓	
Butyric acid			✓				
Lactose			limited	limited		✓	
Oligosaccharides				✓	✓		
Nucleosides						(?)	
Fe	(?)	(?)		✓			
Zn	(?)	(?)					
Se	(?)	✓	✓				

### Relationships between dietary fat and human health

The links between dietary fat and incidence of non-communicable diseases such as coronary heart disease (CHD) and stroke are well established. However, the qualitative composition of fats in the diet has a significant role in modifying the risk, and this has resulted in specific guidelines for intake of different fat types. It is suggested that the contribution of fat and saturated fatty acids (SFA) to dietary energy intake should not exceed 0.35 and 0.1 of total intake, respectively. The ratio of polyunsaturated to saturated fatty acids (P:S ratio) should be around 0.4, and the ratio of *n*-6 to *n*-3 polyunsaturated fatty acids (PUFA) should be less than 4 (Department of Health, 1994; Leaf *et al.*, 2003; Simopoulos, 2001). Ruminant fat typically contains a high proportion of SFA (as a consequence of microbial biohydrogenation within the rumen) and monounsaturated fatty acids (MUFA) and small amounts of PUFA (see below). Linoleic and  $\alpha$ -linolenic acids are the main PUFA while oleic acid (18:1*n*-9) is the most prominent MUFA, with the remainder of the MUFA occurring mainly as *cis* and *trans* isomers of 18:1. The PUFA and MUFA are generally regarded as beneficial for human health and there is even recent evidence of beneficial effects of 18:1 *trans*-11 (Corl *et al.*, 2003), though other work suggests negative effects (Clifton *et al.*, 2004). The predominant SFA are 14:0, 16:0 and 18:0. There are concerns about the effects of SFA of plasma cholesterol, though 18:0 is regarded as neutral in this regard (Yu *et al.*, 1995) and 16:0 is not hypercholesterolemic if the diet contains high levels of linoleic acid (18:2*n*-6; Clandinin *et al.*, 2000). Myristic acid (14:0) is regarded as more potent than palmitic acid (16:0) in raising plasma lipids (Zock *et al.*, 1994). Meat and milk products from ruminants are also the main

dietary sources of conjugated linoleic acid (CLA; Ritzenthaler *et al.*, 2001), which have been identified as possessing a range of health promoting biological properties including anticarcinogenic activity of the dominant CLA in milk and meat, the *cis*-9, *trans*-11 isomer. Research has focused much attention on methods of enhancing the nutritional value of milk and meat by decreasing their content of SFA and increasing *n*-3 PUFA and CLA and assessing the implications for other aspects of product quality.

### **Milk and meat quality and characteristics that influence processability**

Milk fat is an important source of dietary nutrients and energy (Chen *et al.*, 2004). The diet of the animal is the primary determinant of the fatty acid composition of milk and it is evident that milk fatty acid composition can influence technological qualities of milk and milk products including 'softness', shelf life and flavour. The effects of fatty acids on softness (and hence for example spreadability of butter) is due to the different melting points of the fatty acids in milk. As unsaturation increases, melting point decreases. Hence dairy products from cows fed diets rich in unsaturated fatty acids are softer and less viscous and those rich in PUFA are very fluid at higher temperatures, which affect physical structure (Chen *et al.*, 2004). The effects of fatty acids on shelf life are related to the tendency of unsaturated fatty acids to oxidise, leading to rancidity as storage or display time increases. Hence, milk containing higher levels of PUFA is prone to oxidation and addition of antioxidants such as vitamin E is important to limit the development of oxidation in dairy products. Oxidised milk and milk products are characterised by metallic, cardboardy or stale flavours and a paler colour (Timmons *et al.*, 2001). For meat, colour is an important purchase decision by consumers, while tenderness, juiciness and flavour are important characteristics of the eating experience and all may be influenced by the diet of the animal. Total fat rather than individual fatty acids is considered to influence tenderness and juiciness (Wood *et al.*, 2003). The colour of adipose tissue largely reflects the concentrations of  $\beta$ -carotene and lutein. The colour of muscle largely reflects the concentration (and oxidation state) of myoglobin. Thus, when beef is initially cut, the myoglobin oxidises, giving rise to a bright red colour. On exposure to air, its colour changes slowly to brown due to conversion of myoglobin to metmyoglobin. The rate of loss of the more desirable red colour is related to degree of unsaturation of lipids and presence of antioxidants, supplied through the animal's diet or added at processing. Disruption of the tissue during processing provides an additional challenge to the colour and lipid stability of the resulting meat product. As for milk, fatty acids in meat influence flavour. This is related to the production of volatile, odorous, lipid oxidation products during cooking linking with Maillard reaction products to form other volatiles which contribute to aroma and flavour (Wood *et al.*, 2003).

### **The fatty acid composition of milk and beef**

Lean beef has an intramuscular fat content of around 5% with on average 0.45 - 0.48, 0.35 - 0.45 and up to 0.05 of total fatty acids as SFA, MUFA and PUFA, respectively (Moloney *et al.*, 2001). Of the total SFA, 0.3 are represented by stearic acid (18:0). The P:S ratio for beef is typically low at around 0.1 (Scollan *et al.*, 2001), except for double muscled animals which are very lean (<1% intramuscular fat) where P:S ratios are typically 0.5-0.7 (Raes *et al.*, 2001). The *n*-6:*n*-3 ratio for beef is beneficially low, typically less than 3. This reflects the considerable amounts of beneficial *n*-3 PUFA in beef, particularly  $\alpha$ -linolenic (18:3*n*-3) and the long chain PUFA, eicosapentaenoic acid (EPA; 20:5*n*-3) and, docosahexaenoic acid (DHA; 22:6*n*-3). For many people, meat is a significant source of *n*-3 PUFA (British Nutrition Foundation, 1999). Milk fat generally contains 0.6 - 0.7, 0.25 - 0.35 and up to 0.05 as SFA, MUFA and PUFA,

respectively (Jensen, 2002). Linoleic and  $\alpha$ -linolenic acids are the main PUFA - typically 0.02 and 0.005 of milk fatty acids. Oleic acid is on average 0.65 of the MUFA (0.2 of total fatty acids), with the remainder of the MUFA mainly *cis* and *trans* isomers of 18:1.

The following sections will focus on manipulating the beneficial fatty acids in milk and beef by enhancing 18:3*n*-3, CLA and trans-vaccenic acid (18:1 *trans*-11; TVA). The main approach is by changing dietary ingredients which are known sources of long chain PUFA, such as 18:3*n*-3, 20:5*n*-3 and 22:6*n*-3. There are four main sources of fatty acids in ruminant diets: (1) fresh and ensiled forages, (2) oils and oilseeds, (3) fish oil and marine algae and (4) fat supplements. Green plants are the primary source of *n*-3 fatty acids, and forages such as grass and clover contain a high proportion (0.5-0.75) of total fatty acids as  $\alpha$ -linolenic acid (18:3*n*-3). Hence for animals fed on high proportions of forage in the diet, this represents an important source of fatty acids.

### **Increasing the polyunsaturated fatty acid content of milk**

Levels of PUFA in milk are usually very low, though this is a direct consequence of rumen biohydrogenation, and it is feasible to obtain much higher levels if biohydrogenation can be avoided. Feeding high levels of encapsulated sunflower oil led to milk with 35 g/100g of fatty acids as 18:2*n*-6 (Chilliard *et al.*, 2000), whilst feeding very high levels of a product in which linseed oil was protected with formaldehyde-treated proteins led to milk with 20 g/100g of milk fat as 18:3*n*-3 (McDonald & Scott, 1977). Petit *et al.*, (2002) obtained milk with 14 g/100g of milk fat as 18:3*n*-3 by duodenal infusion of linseed oil. A number of protected oilseed products have been fed to dairy cows at a range of levels, resulting in a series of levels of 18:3*n*-3. For example, Goodridge *et al.* (2001) obtained milk with 6.4 g/100g of milk fat as 18:3*n*-3 using a protected linseed product, whilst a poorly-protected product fed by Petit *et al.*, (2002) resulted in only 2.0 g/100g as 18:3*n*-3. Processing oilseeds is generally far less effective at increasing 18:2*n*-6 and 18:3*n*-3 in milk than feeding rumen protected lipid supplements (Kennelly, 1996). The efficiency of transfer of long-chain omega-3 fatty acids from fish oil (Offer *et al.*, 1999) or algal isolates (Offer *et al.*, 2001) is also low.

The highest recorded levels of CLA (5.1% of milk fatty acids) and TVA (17.0% of milk fatty acids) in cows' milk were observed by Gulati *et al.* (2003), who fed 1.1 kg/day of a mixture of soyabean oil and fish oil mixture (70/30) in which the rumen protection technology was weak. This milk also contained an exceptionally low level of 18:0 (2.8% of milk fatty acids). More typical levels of fish oil supplementation (250 g/day) led to CLA and TVA levels of 1.55 and 7.50% of milk fatty acids respectively (Offer *et al.*, 1999).

A number of studies have identified the general relationship between levels of 18:3*n*-3 in herbage and levels of 18:3*n*-3 and CLA in milk. Thomson & Van Der Poel (2000) showed this effect in relation to changes in concentrations of fatty acids in grasses over the grazing season, whilst Chouinard *et al.* (1998) showed effects of the stage of growth at which grass was cut for silage-making. However, Loyola *et al.* (2002) showed differences in the CLA content of milk from cows grazing different ryegrass cultivars, despite their similar fatty acid profiles.

Some of the most marked effects of forages on milk PUFA result from feeding fresh forage as opposed to conserved hay or silage; Tables 2 and 3 provide a summary of reported effects of fresh herbage on 18:3*n*-3 and CLA, respectively. Many of these effects reflect the loss of PUFA during field wilting (see below). The highest levels of 18:3*n*-3 and CLA in milk from pasture-fed cows were 2.31 and 2.21% of milk fatty acids respectively. The level of 18:3*n*-3

achieved with pasture feeding was only around one-tenth of that achieved under extreme conditions, and one-third of levels achieved with a more normal level of protected fat supplement. The level of CLA in milk that was achieved by pasture feeding was comparable to levels achieved with safe levels of fish oil (200-300 g/day).

**Table 2** Effect of the forage component of diets on the  $\alpha$ -linolenic acid (18:*n*-3) content of milk fat (g/100g total fatty acids)

	Diets based on:	
	Fresh forage	Conserved forage
Timmen & Patton (1988)	0.84 (pasture)	0.36 (grass/wheat silage)
Aii <i>et al.</i> (1988)	1.97 (grass)	1.46 (grass hay)
Aii <i>et al.</i> (1988)	1.34 (grass)	1.13 (grass hay)
Hebeisen <i>et al.</i> (1993)	2.31 (grass)	0.45 (conserved grass)
Kelly <i>et al.</i> (1998)	0.95 (grass-white clover)	0.25 (maize and legume silages)
Dhiman <i>et al.</i> (1999)	2.02 (grass-white clover)	0.81 (lucerne hay; grass-white clover)
White <i>et al.</i> (2001)	0.73 (grass-white clover)	0.37 (maize and lucerne silages)
Schroeder <i>et al.</i> (2003)	0.57 (winter oat pasture)	0.07 (maize silage)
Whiting <i>et al.</i> (2004)	1.13 (lucerne)	0.83 (lucerne silage)

**Table 3** Effect of the forage component of diets on the conjugated linoleic acids<sup>1</sup> content of milk fat (g/100g total fatty acids)

	Diets based on:	
	Fresh forage	Conserved forage
Timmen & Patton (1988)	1.34 (pasture)	0.27 (grass and wheat silages)
Precht & Molckentin (1997)	0.76 (grass)	0.38 (maize and grass silages)
Precht & Molckentin (1997)	1.05 (grass)	0.55 (grass silage; green maize)
Kelly <i>et al.</i> (1998)	1.09 (grass-white clover)	0.54 (maize and legume silages)
Dhiman <i>et al.</i> (1999)	2.21 (grass-white clover)	0.89 (lucerne hay; grass-white clover)
White <i>et al.</i> (2001)	0.66 (grass-white clover)	0.36 (maize and lucerne silages)
Schroeder <i>et al.</i> (2003)	1.12 (winter oat pasture)	0.41 (maize silage)

<sup>1</sup>generally *cis*-9, *trans*-11 18:2

A number of applied studies have investigated effects of restricting pasture, and shown either no change or small decreases in levels of 18:3*n*-3 and CLA in milk when pasture allocation is reduced (Stanton *et al.*, 1997; Stockdale *et al.*, 2003). Loor *et al.* (2003) showed 40-70% increases in levels of 18:3*n*-3, CLA and TVA in milk when cows were grazed for 7-8 hour periods in addition to being fed conserved forages in a total mixed ration (TMR). Alpine pasture leads to production of milk (cheese) with enhanced levels of CLA and *n*-3 PUFA (Innocente *et al.*, 2002; Hauswirth *et al.*, 2004). Collomb *et al.* (2002) investigated these effects further and identified relationships between milk fatty acids and the species contained within herb-rich pastures. A number of these associations merit further attention in terms of potential mechanisms for increasing milk PUFA.

Recent studies with *Trifolium pratense* L. (red clover) silage (Dewhurst *et al.*, 2003; Lee *et al.*, 2003) have identified a substantial reduction in the extent of rumen biohydrogenation of

18:3*n*-3 when feeding *T. pratense* silage as opposed to grass silage. This effect relates, in part at least, to the effect of polyphenol oxidase (PPO), which is activated when *T. pratense* tissue is damaged, reducing the extent of lipolysis (Lee *et al.*, 2004).

There is limited evidence of potential for production of 20:5*n*-3 by fatty acid chain elongation from (forage) 18:3*n*-3 in the mammary gland (Hebeisen *et al.*, 1993). Increasing supply of the precursor, 18:3*n*-3 does not always increase 20:5*n*-3 (Petit *et al.*, 2002; Whiting *et al.*, 2004).

### Increasing the polyunsaturated fatty acid content of beef

Increasing the P:S ratio, reducing the *n*-6:*n*-3 ratio and increasing CLA are important targets for research aimed at improving the nutritional value of beef. In general, nutritional manipulation does not increase the P:S ratio in the meat above the normal range (0.06-0.15), reflecting the high degree of biohydrogenation of dietary PUFA in the rumen. Providing equivalent amounts of 18:3*n*-3 as linseed in the diet (L) or linseed oil (LO) into the small intestine increased the percentage and amount (mg/100 g muscle) of 18:3*n*-3 in total lipid to 1.0, 1.5 and 8.7 and 15.8, 26.3 and 176.5 for the control, L and LO, respectively (Gatellier *et al.*, 2004). The LO treatment resulted in a high P:S ratio (0.495 relative to the recommended target of > 0.4) and low *n*-6:*n*-3 ratio (1.04 relative to the recommended target of < 2-3). This demonstrates the high potential to deposit 18:3*n*-3 in muscle lipids. Similarly, using ruminally protected lipids (rich in both 18:2*n*-6 and 18:3*n*-3) resulted in meat characterised by higher P:S ratio (0.22), lower *n*-6:*n*-3 ratio (1.8) and 3.0% 18:3*n*-3 (Scollan *et al.*, 2004).

Feeding pasture relative to concentrates, rich in 18:3*n*-3 and 18:2*n*-6, respectively, results in higher concentrations of *n*-3 PUFA in muscle lipids (French *et al.*, 2001; Nuernberg *et al.*, 2004). Grass relative to concentrate feeding not only increased 18:3*n*-3 in muscle phospholipid but also 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 (Warren *et al.*, 2002). Concentrates rich in 18:2*n*-6 lead to higher concentrations of 18:2*n*-6 and associated longer chain derivatives (20:4*n*-6). French *et al.* (2001) demonstrated that an increase in the proportions of grass in the diet decreased SFA concentration, increased P:S and *n*-3 PUFA concentration and decreased *n*-6:*n*-3 PUFA. These beneficial responses with grass have been related to time at pasture (Table 4; Noci *et al.*, 2003). Feeding mixtures of grass and clover (both white and red clover) relative to grass alone increased the deposition of both *n*-6 and *n*-3 PUFA in muscle of finishing beef steers, contributing to increases in the P:S ratio (Scollan *et al.*, 2002).

**Table 4** Nutritionally important fatty acids of *longissimus thoracis* muscle in heifers fed on grass for differing times (Noci *et al.*, 2003)

% of muscle fatty acids	Days at grass				s.e.d.	P <sup>1</sup>
	0	40	99	158		
Sum SFA	45.4	45.8	45.5	43.2	0.77	**L,Q
TVA	1.35	1.93	2.27	3.01	0.18	L
CLA cis-9, trans-11	0.50	0.50	0.57	0.71	0.06	***L
Sum <i>n</i> -6 PUFA	3.25	3.20	2.97	3.31	0.23	NS
Sum <i>n</i> -3 PUFA	1.79	2.06	1.91	2.43	0.17	**L
<i>n</i> -6: <i>n</i> -3 ratio	2.00	1.79	1.56	1.32	0.10	***L
P:S ratio	0.12	0.14	0.12	0.15	0.009	*

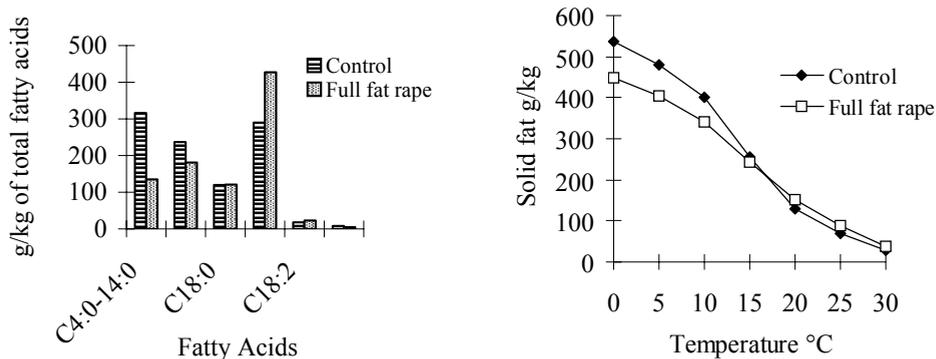
<sup>1</sup>L and Q are significant linear and quadratic effects of days at grass, respectively; SFA = saturated fatty acids.  
\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

The main CLA isomer in beef is CLA *cis*-9, *trans*-11 and it is mainly associated with the neutral lipid fraction (typically 92% of total CLA in muscle lipid) and hence is positively correlated with fatness. As with milk, CLA in tissue is influenced by (1) CLA and TVA produced in the rumen, and (2) conversion of TVA to CLA in the tissue. Moloney *et al.* (2001) have reviewed the concentrations of CLA in beef across a range of production systems and found values ranging from 1.2-12.5 mg/g fat. Supplementing diets with sunflower oil or seeds, linseed or soya bean oil increases CLA and TVA in beef (Mir *et al.*, 2003). Grass relative to concentrate feeding, results in high CLA, and a positive association between CLA content and duration at pasture before slaughter has been reported (Table 4).

### **Relationship between fatty acid composition of milk and processability**

The fatty acid composition of milk has a large impact on quality and processability characteristics. Feeding formaldehyde treated oilseeds to help reduce ruminal biohydrogenation of dietary lipids, resulted in milk fat containing up to 350 g/kg of 18:2*n*-6 and 220 g/kg of 18:3*n*-3 (Cook *et al.*, 1972; Bitman *et al.*, 1975; Wrenn *et al.*, 1976). This milk fat was highly susceptible to oxidation and had an unsatisfactory melting profile with the fat becoming liquid at about 20 °C. Diets that increased the proportion of oleic acid (18:1*n*-9) in milk also generally decrease the proportion of 16:0, resulting in 'softer' milk fat with a more desirable melting profile. The results in Figure 1 illustrate the effect of feeding approximately 700 g/day of rapeseed oil, as processed full fat rapeseeds, on the fatty acid composition and the resulting melting profile of the milk fat. The short to medium chain fatty acids and 16:0 were reduced proportionally by 0.40 and 0.24, respectively, while 18:1*n*-9 was increased by 0.47. These fatty acid changes resulted in a solid fat at 10°C of 341 g/kg compared to 401 g/kg in the milk fat from unsupplemented cows.

Comparing milk fat from cows fed control diets indoors and at pasture, showed that milk fat produced at pasture was proportionally lower in 16:0 and higher in 18:1*n*-9 by 0.19 and 0.55, with solid fat contents at 10°C of 520 and 401 g/kg, respectively (Murphy, 2000). Similar seasonal variation in the softness/spreadability of butter was observed in New Zealand (MacGibbon & McLennan, 1987) and related to variation in milk PUFA (Thomson & Van der Poel, 2000). These data highlight the enhanced fatty acid composition of milk fat produced at pasture and the opportunity to manipulate composition in a beneficial way by relatively simple supplementation with whole oilseeds containing high proportions of 18-carbon fatty acids. 'Spreadable' butter is being produced commercially in Northern Ireland using this strategy and factors that influence the consistency of the fat produced are being investigated (Magowan *et al.*, 2003, 2004). Milk having a high level of PUFA is more susceptible to autoxidation than conventional milk. Production of oxidised flavour at 8 days post sampling was positively correlated with levels of 18:2*n*-6 ( $r=0.49$ ), 18:3*n*-3 ( $r=0.55$ ) and total PUFA ( $r=0.50$ ) in milk fat (Timmons *et al.*, 2001). Milk for cows fed on *T. pratense* compared to grass silage contained more 18:2*n*-6 and 18:3*n*-3 PUFA which resulted in increased oxidative deterioration of milk (Al-Mabruk *et al.*, 2004). The latter could be corrected by feeding supplemental vitamin E. The taste of milk from diets containing *T. pratense* was negatively affected (Bertilson & Murphy, 2003).



**Figure 1** The effect on milk fatty acid composition and resulting melting profile of the milk fat of feeding approximately 700 g/day of rapeseed oil, in the form of processed full fat rapeseeds, to dairy cows at pasture (Murphy, 2000)

### Relationship between fatty acid composition of meat and processability

Differences between the quality characteristics of meat from grass-fed or non-grass (usually grain)-fed cattle or sheep have been reviewed (Muir *et al.*, 1998; Priolo *et al.*, 2001; Geay *et al.*, 2001). In general there were no consistent effects of these feeds on meat colour, pH, drip loss, water holding capacity, cooking loss, tenderness or juiciness. Grazing consistently results in an increase in yellowness of fat (Muir *et al.*, 1998), reflecting the higher concentrations of  $\beta$ -carotene and lutein in grass compared to alternative feedstuffs.

Even though pasture feeding results in higher concentrations of more oxidisable *n*-3 PUFA in muscle lipids, the meat is more resistant to lipid oxidation than grain fed-beef (O'Sullivan *et al.*, 2003). This reflects the higher deposition of plant derived anti-oxidants, in particular vitamin E, in meat from pasture-fed cattle, but also increased activity of some anti-oxidant enzymes (Gatellier *et al.*, 2004). This observation generally holds for fresh or aged meat (Yang *et al.*, 2002) and meat that has undergone long-term frozen storage (Farouk & Wieliczko, 2003), but the opposite may occur when this meat is minced (Realini *et al.*, 2004). The authors suggest that mincing disrupts cellular integrity and exposes more of the polyunsaturated fatty acids to oxidation - providing a greater test of anti-oxidative protection of the *n*-3 PUFA in grass-fed beef. Similarly, Yang *et al.*, (2002) reported that at similar vitamin E concentrations, pasture-fed beef was less stable than concentrate beef, again highlighting the influence of the fatty acid composition of pasture-fed beef. The appropriate ratio of vitamin E (and other antioxidants) to *n*-3 PUFA in meat to ensure lipid and colour stability during processing remains to be determined. With regard to general functional properties, Farouk & Wieliczko (2003), concluded that there is not much difference between beef finished on pasture or grain even after long-term frozen storage.

In a recent review examining the relationships between fatty acids and meat quality, it was concluded that only when concentrations of linolenic acid approach 3% of lipids are there any adverse effects on lipid stability, colour stability or flavour. In support of this conclusion, when the concentration of linolenic acid in beef was increased from 0.7% to 1.2% of total lipids by feeding linseeds (Vatansever *et al.*, 2000), or to 1.9% by using a ruminally-protected

lipid supplement (Scollan *et al.*, 2003; Enser *et al.*, 2001), there was little effect on lipid stability or flavour characteristics. When linolenic acid was increased to 2.8% of total fatty acids, higher sensory scores for 'abnormal' and 'rancid' were recorded (Scollan *et al.*, 2004).

Since grazing *per se* does not increase the proportion of linolenic acid to the extent achieved by the use of protected lipid supplements, little difference between fatty acid-derived flavours might be expected between grass-fed and grain-fed beef. Muir *et al.*, (1998) concluded that on the basis of similar weight or fat cover, the differences resulting from these feeding regimes were not sufficient to generate differences in flavour 'factors' which were large enough to be detected by the sensory panellists. Clearly much of the perception of differences between grass and grain-fed beef flavour is confounded by other factors, in particular fatness, which contributes to flavour. Moreover, when preferences for a particular product are expressed, they may be influenced by prior experience (Sanudo *et al.*, 1998). Non-lipid compounds and their metabolites, derived from pasture constituents may also contribute directly to flavour. Thus, compared with a grain diet, grass feeding increased the concentration of diterpenoids (which derive from chlorophyll breakdown in the rumen) (Melton, 1990) and 3-methylindole (skatole) (Young *et al.*, 1997).

Little has been published on differences in the quality of beef from cattle fed different forages. Scollan *et al.*, (2002) found little difference in the proportions of 18:2 or 18:3 in total lipids of beef from cattle fed grass or mixtures of grass and red or white clover, and no difference in colour stability, lipid stability or flavour. Wilting grass prior to ensiling did not change the quality characteristics of beef compared to beef from cattle fed unwilted grass silage from a similar sward (Moloney *et al.*, 2004). In contrast, extensive rather than restricted fermentation in the silo resulted in beef that was more colour stable and visually more desirable (O'Sullivan *et al.*, 2004). This reflected differences in vitamin E concentrations indicating that conservation strategies should consider loss of antioxidants as well as PUFA.

The effects of different forages on the quality of lamb have been recently reviewed (Duckett & Kuber, 2001). These authors concluded that compared to grazed grass, the intensity of flavour in lamb is increased with grazing of *Trifolium repens* L. (white clover), *Medicago sativa* (lucerne), and certain crop aftermaths, but that these differences can be decreased by grazing grass for 2 to 3 weeks before slaughter. In contrast, Vipond *et al.* (1995) failed to demonstrate an effect of clover on the flavour of lamb. They suggested that differences between the experiments might be due to the proportion of clover in the diet, in that many earlier studies used pure swards in their comparisons, which are unlikely to be used in commercial practise. There also seems to be seasonal effects on flavour intensity with maximum intensity occurring in meat from lambs grazing in cooler months (Duckett & Kuber, 2001). Young *et al.* (1994) reported that feeding lambs on a range of different pasture species resulted in different sensory attributes of the meat.

### **Breeding and managing forage for fatty acids**

The transfer of forage linolenic acid to milk or meat is dependent on two important processes, (1) increasing the level of 18:3 $n$ -3 in the feed and (2) reducing the extent of biohydrogenation. The former is dependent on maximising levels in the fresh herbage, and for silage reducing losses during field wilting. A number of authors (Dewhurst *et al.*, 2001; Elgersma *et al.*, 2003a; Boufaied *et al.*, 2003) have recently commented on genetic variation in herbage fatty acid levels. Quantitative Trait Loci (QTL) have been identified in a ryegrass mapping family,

which will facilitate rapid selection for high-lipid grasses. As with most herbage quality traits, there are substantial environmental effects and interactions that make exploitation more difficult. The most notable effects are the number and timing of cuts and season (Saito *et al.*, 1969; Bauchart *et al.*, 1984; Dewhurst *et al.*, 2001; Boufaied *et al.*, 2003; Elgersma *et al.*, 2003a,b). Management which inhibits the initiation of flowering (e.g. two early cuts in the work of Bauchart *et al.*, 1984 and 9-cuts per annum in the work of Dewhurst *et al.*, 2002) increases fatty acid levels.

Oxidative loss of PUFA during field wilting represents a major loss to the food chain, with substantial losses of 18:3 $n$ -3 during hay-making (Aii *et al.*, 1988), and modest losses during wilting prior to ensiling (Dewhurst & King 1998; Boufaied *et al.*, 2003). These losses are associated with the lipoxygenase system - a plant defence mechanism which is initiated in damaged tissues. Plant lipases release free 18:3 $n$ -3 and 18:2 $n$ -6 from damaged membranes (Thomas, 1986), and these are rapidly converted to hydroperoxy PUFA by the action of lipoxygenases (Feussner & Wasternack, 2002). The hydroperoxy PUFA are further catabolised to yield a range of volatile anti-microbial and anti-fungal compounds, such as leaf aldehydes and alcohols (Fall *et al.*, 1999). These compounds develop rapidly and provide the smell of freshly cut herbage. 'Stay-green' grasses have provided one approach to reducing wilting losses. 'Stay-green' grasses, which lack one of the enzymes involved in chlorophyll breakdown (Thomas & Smart, 1993) and so retains thylakoid membrane structure later in senescence, have been investigated. Stay-green material showed substantially reduced losses of fatty acids when artificially senesced by excision and incubation on moist filter paper in darkness (Harwood *et al.*, 1982). Dewhurst *et al.* (2002) found a small reduction in losses of fatty acids during wilting, though the effect may have been restricted by the rapid drying conditions.

### **Rumen biohydrogenation**

The extent of biohydrogenation of dietary PUFA is very high, averaging approximately 86 and 92% for 18:2 $n$ -6 and 18:3 $n$ -3, respectively. However, this process does give rise to a number of metabolically important intermediates including 18:1 $trans$  isomers and CLA, both of which determinant the levels of CLA in milk and meat. Whilst forages usually represent the main source of 18:3 $n$ -3 in the diet, this tends to be counteracted by increasing biohydrogenation with increasing forage proportion in the diet (Kalscheur *et al.*, 1997; Kucuk *et al.*, 2001), reflecting the predominant role of the fibrolytic bacterium *Butyrivibrio fibrisolvens* in this process (Demeyer & Doreau, 1999).

As discussed, high levels of 18:3 $n$ -3 may be achieved in milk and meat by using ruminally protected lipids, hence reducing the extent of biohydrogenation. Establishing natural methods of modifying the extent of biohydrogenation and generating important biohydrogenation intermediates is an important target for on-going research. For forages, promising results have appeared for *T. pratense* expressing high PPO activity. Studies also suggest that forages expressing high PPO activity may reduce lipid losses in the rumen (Lee *et al.*, 2004). *In vivo* studies have demonstrated a lower degree of biohydrogenation on *T. pratense* compared with grass, and beneficially higher flows of linolenic acid (18:3 $n$ -3) to the duodenum (Lee *et al.*, 2003).

## Use of existing knowledge now

Increased health consciousness among consumers has led to a growing preference for healthier, more nutritious and more functional food products. It is evident that milk and meat contain components which offer beneficial effects for human health, and which are improved by grass feeding. This provides a basis on which to develop a range of novel 'functional foods'. The term functional foods is a generic term used to describe foods or food components that have beneficial effects on human health above that expected on the basis of nutritive value (Milner, 1999). Such products are targeted at disease prevention and are aimed at healthy people. Milk and meat and associated products offer exciting opportunities in this rapidly developing area, and the functional food components in milk and meat help to illustrate the important role of these products in the human diet. Indeed in some countries, omega-3 enriched milk and dairy products are on the market. The meat industry has been the slowest section of the food industry to embrace the functional trend and incorporate functional ingredients into their products, but this is changing. Grassland production systems have the potential to enhance the content of beneficial fatty acids in meat and milk, improve stability (from higher antioxidant content) and alter sensory attributes. These attributes offer considerable scope to help create product differentiation in increasingly competitive markets.

## Future research

Exploitation of forage based systems as a route to the production of meat and milk and associated products with enhanced product quality characteristics offers outstanding opportunities. However, such systems do present significant challenges and research must address a number of issues to help ameliorate particular problems. Some of these are discussed below:

1. Variability in both quality and quantity of forage represents a major challenge for these systems, which can lead to variability in product quality. Traditionally producers have compensated for variations in nutrients supply from forage by the strategic use of concentrates. Season, drought and flowering all have large effects on forage characteristics. Extensive forage systems that consistently deliver (all-year-round) high quality milk and meat are an important target. More attention will need to be given to the appropriate choice of animal genotype to fit these systems.
2. Development of future forage genotypes should emphasise characteristics that facilitate consistency of supply and effects of quality, including fatty acids, shelf life/stability and sensory attributes of meat and milk products. Progress should continue in improving intake and digestibility characteristics of forages, but efforts, for example to increase the lipid content (and proportion of 18:3 $n$ -3) through genetic selections based on QTL offer scope for reasonably fast progress. Recent transgenic research has reported the production of longer chain  $n$ -6 and  $n$ -3 PUFA in higher plants (*Arabidopsis thaliana*; Qi *et al.*, 2004). There is considerable scope for similar approaches in grasses as an alternative and more sustainable route to enhancing delivery of longer chain PUFA into the food chain than using fish oils.
3. Biohydrogenation of dietary PUFA by ruminal micro-organisms is a major limitation to our ability to beneficially enhance the fatty acid composition of ruminant products. Understanding the major microbial species involved in biohydrogenation and how they are influenced by diet will permit an increased understanding of methods of modifying this process.

4. Grass feeding may have some beneficial effects on improving lipid and colour stability of raw meat and milk, but this will merit further attention when these materials are processed further. In this respect, the relationships between vitamin E (and other antioxidants) and *n*-3 PUFA in meat and milk to ensure adequate stability during processing require attention.
5. The relationships between pasture species, and in particular herb-rich pastures and fatty acid composition and sensory attributes of meat and milk is interesting, and further studies here may reveal useful mechanisms for increasing the quality of ruminant products. Greater integration of research across the various levels of the food chain and increased cooperation with industry will aid the development of foods with higher quality and safety together with clear health benefits for consumers.

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