Enrichment of ruminant products with nutritionally beneficial fatty acids

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Summary
Consumers are increasingly becoming more aware of the relationships between diet and health. The functional–food components of beef and milk fat include ω–3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA). Beef typically has high saturated fatty acid content and low PUFA content, but the content of beneficial fatty acids can be increased by dietary manipulation. Dietary linseed (rich in ω–3 PUFA α–linolenic acid, 18:3n–3) can double the contents of 18:3n–3 and eicosapentaenoic acid (EPA, 20:5n–3) in muscle and adipose tissue, resulting in a lower n–6:n–3 ratio. Beef from grass–fed animals has higher levels of 18:3n–3, EPA and docosahexaenoic acid (22:6n–3) than that from concentrate–fed animals. Protection of dietary PUFA from ruminal biohydrogenation results in further enhancement of the PUFA content of meat. The main CLA isomer in beef is cis–9, trans–11 CLA, which is mainly associated with the neutral lipid fraction; the concentration of this isomer is thus positively correlated with the degree of fatness. PUFA–rich diets increase the content of cis–9, trans–11 CLA in beef. As the dietary content of n–3 PUFA increases, undesirable sensory attributes such as ‘greasy’ and ‘fishy’ increase and colour and shelf life may be reduced, necessitating the use of higher levels of dietary antioxidants. These nutritional strategies provide mechanisms for increasing the content of health–promoting fatty acids in beef.

Keywords: health, nutrition, fatty acids, beef

Introduction
The relationship between dietary fat intake and the incidence of lifestyle diseases, particularly coronary heart disease, is well established and has contributed to the development of specific government health recommendations for food components, especially fats (Simopoulos 2001). It is recommended that the contribution of fat and saturated fatty acids (SFA) to dietary energy intake should not exceed 0.35 and 0.10 of total intake, respectively, and that the ratio of polyunsaturated to saturated fatty acids (P:S ratio) should be 0.4 and the ratio of n–6 to n–3 polyunsaturated fatty acids (PUFA) should be less than 4 (Simopoulos 2001; Leaf et al. 2003). An increase in intake of ω–3 fatty acids (FA) is considered essential to rectify the imbalance in the ratio of n–6:n–3 PUFA of current Western diets (15:1); the ratio in the diet of primitive man was 1:1, which resulted from the consumption of plant oils rich in linoleic acid (18:2n–6). Of the n–3 PUFA, eicosapentaenoic acid (EPA, 20:5n–3) and docosahexaenoic acid (DHA; 22:6n–3) reduce the risk of cardiovascular disease, cancer and type–2 diabetes, and are critical for proper brain and visual development in the foetus and maintenance of neural and visual tissues throughout life (Leaf et al. 2003). The precursor of the n–3 series is α–linolenic acid (18:3n–3), as man has the capacity to synthesise the C20 PUFA (EPA and DHA) from 18:3n–3. Meat, fish, fish–oils and eggs are important sources of these ω–3 PUFA. As a consequence, opportunities to enhance the content of beneficial fatty acids in various foods, including ruminant products, are being studied.

Ruminant fat is rich in saturated fatty acids, which has contributed towards a negative consumer image of ruminant products during the past 10–15 years. However, these products do contain small amounts of ω–3 PUFA and, for many people, they make an important contribution to nutritional intake when fish consumption is low (British Nutrition Foundation 1999). Meat and milk products from ruminants are also the main dietary sources of conjugated linoleic acid (CLA; Ritzenthaler et al. 2001), which has a range of health–promoting biological properties; the predominant CLA in milk and meat, the cis–9, trans–11 isomer, has anticarcinogenic properties. Research has focused on methods of enhancing the nutritional value of milk and meat by decreasing the content of SFA and increasing n–3 PUFA and CLA content.

This paper focuses on nutritional factors that influence health aspects of beef. A recent review by
The fatty acid composition of beef

Lean beef has an intramuscular fat content of about 5% or less with an average of 0.45–0.48% SFA, 0.35–0.45% MUFA and up to 0.05% PUFA (Moloney et al. 2001). The ratio of polyunsaturated to monounsaturated fatty acids (P:S ratio) for beef is usually about 0.1 (Scollan et al. 2001a), except in double–muscled animals, which are very lean (<1% intramuscular fat) and have a P:S ratio of 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 18:3 n–3 and the long chain PUFA, EPA and DHA.

The predominant saturated fatty acids (SFA) are 14:0 (myristic acid), 16:0 (palmitic acid) and 18:0 (stearic acid). Of the total SFA, 0.3 are represented by 18:0. SFA are known to influence plasma cholesterol, although 18:0 is regarded as neutral (Yu et al. 1995) and 16:0 is not hypercholesterolemic if the diet contains high levels of 18:2 n–6 (Clandinin et al. 2000). Myristic acid is regarded as a more potent acid for raised plasma lipid concentrations than palmitic acid (Zock et al. 1994). Linoleic and α–linolenic acids are the main PUFA. Oleic acid (18:1 n–9) is the most prominent MUFA, 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; there is recent evidence of beneficial effects of 18:1 trans–11 (TVA; Corl et al. 2003), although other work suggests that it has negative effects (Clifton et al. 2004). The main CLA isomer in beef is cis–9, trans–11 CLA, which comprises 72–90% of the total CLA isomer content. Dannenberger et al. (2004) reported 10 isomers of CLA in beef, cis–9, trans–11 CLA representing approximately 70% of total CLA isomers. The trans–11, cis–13, trans–7, cis–9 and trans–10, cis–12 isomers represent approximately 9%, 8% and 2% of total CLA isomers, respectively. Biological effects of cis–9, trans–11 and trans–10, cis–12 CLA have been investigated extensively. The anticarcinogenic and antiatherogenic effects of cis–9, trans–11 and the anti–obesity effects of trans–10, cis–12 have been documented (Belury 2002).

Factors influencing the fatty acid composition of beef

The fatty acid composition of beef is influenced by genetic factors, albeit to a lower extent than dietary factors. De Smet et al. (2004) concluded that breed differences are generally small but nevertheless reflect differences in gene expression or enzymes involved in fatty acid synthesis, and therefore merit attention. Of particular note is the association between fatness and P:S ratio (Figure 1). Because concentrations of saturated and monounsaturated fatty acids increase faster with increasing fatness than the concentration of PUFA, the relative proportion of PUFA and the P:S ratio decrease with increasing fatness.

Choi et al. (2000) examined differences in fatty acid composition between Holstein–Friesians and Welsh Black cattle. Total muscle fatty acid concentrations are higher in Holstein–Friesians than Welsh Blacks (Table 1). That dairy breeds have more marbling fat than beef breeds has been observed by other workers and is often associated with increased tenderness and juiciness in the cooked meat. The concentrations of the three major fatty acids in beef muscle, 16:0, 18:0 and 18:1 n–9, are higher in Holstein–Friesians because of their higher concentration of marbling fat. However, EPA is 20% higher in the Welsh Black. When expressed as a proportion of the total fatty acids, 18:3 n–3 as well as EPA is greater in the Welsh Black. This is related to the higher proportion of phospholipid in the total lipid (rather than neutral lipid). These differences in the content of n–3 PUFA in the Welsh Black resulted in improved P:S and n–6:n–3 ratios compared to those in Holstein–Friesian (0.11 vs. 0.07 and 2.49 vs. 2.00, respectively).
Most research has focused on altering dietary ingredients that are known sources of long chain PUFAs such as 18:1n–9, 18:2n–6, 18:3n–3, EPA and DHA. There are three main sources of fatty acids in the diet of beef cattle: (1) fresh and ensiled forages, (2) oils and oilseeds, (3) fish oil and marine algae. Green plants are the primary source of n–3 fatty acids. Forages such as grass and clover contain a high proportion (50–75%) of total fatty acids as ω–linolenic acid (18:3n–3; Dewhurst and King 1998). Whole oilseeds or seed oils such as rapeseed (rich in 18:1n–9), soyabean and sunflower (rich in 18:2n–6) and linseed (rich in 18:3n–3) have frequently been used to manipulate the fatty acid composition of ruminant products. Fish oils are rich in the long chain PUFA, EPA and DHA. Plants have the unique ability to synthesise *de novo* 18:3n–3, which is the building block of the n–3 series of essential fatty acids. Elongation and desaturation of this fatty acid results in the synthesis of EPA and DHA. The formation of these long chain n–3 PUFA by marine algae and their transfer through the food chain to fish accounts for the high concentrations of these important fatty acids in fish oils.

The inclusion of linseed or linseed oil in a concentrate diet for cattle resulted in increases of acids in fish oils. For the high concentrations of these important fatty acids, their transfer through the food chain to fish accounts of these long chain results in the synthesis of EPA and DHA. The formation of these long chain n–3 PUFA by marine algae and sunflower oils mixed to give a 2.4:1 ratio of 18:2n–6 to 18:3n–3 to beef cattle ingesting grass silage *ad libitum* resulted in meat characterised by a higher content of both 18:2n–6 and 18:3n–3 and a higher P:S ratio relative to meat from cattle fed the control diet. However, there was less improvement in the n–6:n–3 ratio, which depends on the competition between n–6 and n–3 PUFA for deposition in muscle lipids (in particular in phospholipid). This work helped to focus attention on the importance of the ratio of n–6:n–3 ratio in the PLS for optimising the balance of fatty acids deposited. A further study investigated the effect of feeding a PLS with a lower n–6:n–3 ratio (Scollan et al. 2004). Charolais steers were given grass silage *ad libitum* plus one of four concentrates in which the lipid source was either Megalac (Mega, rich in palmitic acid; 16:0) or PLS (soyabean, linseed and sunflower oils resulting in a 1.1:1 ratio of 18:2n–6 to 18:3n–3). Concentrate 1 (Mega, control) contained 139 g/kg Mega, concentrate 2 (PLS1) contained 67 g/kg Mega with 400 g/d PLS fed separately, concentrate 3 (PLS2) contained 24 g/kg Mega with 800 g/d PLS fed separately, and concentrate 4 (PLS3) contained no Mega and 1000 g/d PLS fed separately. Feeding PLS increased the content of 18:2n–6 and 18:3n–3 by a factor

### Table 1 Effect of breed on the fatty acid composition of *Longissimus dorsi* muscle (mg/100 g muscle; Choi et al. 2000).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Holstein–Friesian</th>
<th>Welsh Black</th>
<th>SEM</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>1104</td>
<td>720</td>
<td>99.2</td>
<td>0.01</td>
</tr>
<tr>
<td>18:0</td>
<td>585</td>
<td>460</td>
<td>57.1</td>
<td>NS</td>
</tr>
<tr>
<td>18:1 TVA</td>
<td>109</td>
<td>78</td>
<td>12.6</td>
<td>NS</td>
</tr>
<tr>
<td>18:1n–9</td>
<td>1504</td>
<td>881</td>
<td>148.1</td>
<td>0.01</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>115</td>
<td>93</td>
<td>5.2</td>
<td>0.01</td>
</tr>
<tr>
<td>18:3n–3</td>
<td>34.8</td>
<td>32.9</td>
<td>2.03</td>
<td>NS</td>
</tr>
<tr>
<td>20:5n–3 EPA</td>
<td>14.7</td>
<td>18.0</td>
<td>0.97</td>
<td>0.05</td>
</tr>
<tr>
<td>22:6n–3 DHA</td>
<td>4.0</td>
<td>4.0</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Total fatty acid</td>
<td>4222</td>
<td>2763</td>
<td>525.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>
of 2.3 and 4.2, respectively (Table 2). The content of EPA (synthesised from 18:3n–3) was increased by PLS. The P:S ratio was increased but the n–6:n–3 ratio was reduced by the inclusion of PLS. Thus, feeding a protected lipid supplement with an n–6:n–3 ratio of 1:1 compared to 2:4:1 (Scollan et al. 2003a) resulted in a much lower n–6:n–3 ratio in meat (1.88 vs. 3.59, respectively).

Forages are an important source of n–3 PUFA in the food chain. Feeding forages rich in 18:3n–3 relative to concentrates, which have higher 18:2n–6 concentrations, results in higher concentrations of n–3 PUFA in muscle lipids (Warren et al. 2002; Nuernberg et al. 2004). Feeding grass rather than concentrates not only increased 18:3n–3 in muscle phospholipid but also EPA, DPA and DHA (see Table 3; Warren et al. 2002). Concentrates rich in 18:2n–6 lead to higher concentrations of 18:2n–6 and associated longer chain derivatives (20:4n–6), French et al. (2000a) found that an increase in the proportions of grass in the diet decreased concentrations of saturated fatty acids, increased the P:S ratio and n–3 PUFA concentration, and decreased the n–6:n–3 PUFA ratio.

Subsequently, studies in Ireland showed that these beneficial responses with grass were related to time at pasture (Noci et al. 2004). Feeding mixtures of grass and clover (both white and red clover) increased the deposition of both n–6 and n–3 PUFA in muscle of finishing beef steers relative to that obtained by feeding grass alone, resulting in a increase in the P:S ratio (Scollan et al. 2002).

There is much interest in conjugated linoleic acid (CLA). Our research has confirmed that the main CLA isomer in beef is cis–9, trans–11 CLA, which is mainly associated with the neutral lipid fraction (typically 92% of total CLA in muscle lipid) and is thus positively correlated with the degree of fatness. It is also established that the majority of CLA found in muscle is synthesised from TVA via delta–9 desaturase in the tissue rather than being derived directly from the rumen. In general, feeding PUFA–rich diets (i.e., sunflower oil, soya, linseed or fish oil) results in increases in tissue CLA content (Mir et al. 2003). Pasture grazing also results in higher CLA content and there is a positive association between CLA content and duration at pasture before slaughter (Noci et al. 2004).

The increases in n–3 PUFA and CLA content of beef associated with forage feeding are very beneficial. Current research at the Institute of Grassland and Environmental Research (IGER) is investigating forage factors and forage management strategies that influence the delivery of n–3 PUFA from the forage through to the product. The latter is dependent on two important processes: (1) increasing the level of 18:3n–3 in the feed and (2) reducing the extent of biohydrogenation in the rumen. Studies at IGER have identified considerable genetic variation in herbage fatty acid levels, which provides opportunities for breeding high–lipid grasses (Dewhurst et al. 2001).

It is also known that fatty acid levels may vary with season and are influenced by the number and timing of cuts (Dewhurst et al. 2001). For hay and silage making, large oxidative losses of PUFA may occur during field wilting (Dewhurst and King 1998).

**Biohydrogenation**

Dietary PUFA are rapidly hydrogenated by rumen microbes, resulting in the production of saturated fatty acids (principally 18:0) and formation of CLA and trans monoene intermediates (Demeyer and Doreau 1999). This is one of the main reasons why ruminant fats are highly saturated. The extent of biohydrogenation influences the amount of saturated fatty acids produced in the rumen and the amounts of CLA and TVA. The

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**Table 2** Effect of feeding ruminally protected lipid supplements (PLS) on the fatty acid composition (mg/100 g muscle) of longissimus dorsi (Scollan et al. 2004).

<table>
<thead>
<tr>
<th></th>
<th>Mega</th>
<th>PLS1</th>
<th>PLS2</th>
<th>PLS3</th>
<th>SED</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>1305</td>
<td>1311</td>
<td>1238</td>
<td>1175</td>
<td>205.2</td>
<td>NS</td>
</tr>
<tr>
<td>18:0</td>
<td>717</td>
<td>731</td>
<td>773</td>
<td>788</td>
<td>114.2</td>
<td>NS</td>
</tr>
<tr>
<td>18:1 TVA</td>
<td>105.2</td>
<td>110.7</td>
<td>113.2</td>
<td>132.5</td>
<td>18.43</td>
<td>NS</td>
</tr>
<tr>
<td>18:1n–9</td>
<td>1645</td>
<td>1669</td>
<td>1610</td>
<td>1612</td>
<td>275.9</td>
<td>NS</td>
</tr>
<tr>
<td>18:1 cis</td>
<td>68.1</td>
<td>68.8</td>
<td>65.9</td>
<td>66.9</td>
<td>10.11</td>
<td>NS</td>
</tr>
<tr>
<td>18:2n–6</td>
<td>120.4</td>
<td>255.2</td>
<td>279.0</td>
<td>305.1</td>
<td>23.36</td>
<td>0.001</td>
</tr>
<tr>
<td>18:3n–3</td>
<td>28.6</td>
<td>101.7</td>
<td>117.9</td>
<td>138.9</td>
<td>13.08</td>
<td>0.001</td>
</tr>
<tr>
<td>cis–9, trans–11 CLA</td>
<td>19.9</td>
<td>24.2</td>
<td>24.1</td>
<td>31.0</td>
<td>4.29</td>
<td>NS</td>
</tr>
<tr>
<td>20:5n–3 EPA</td>
<td>12.6</td>
<td>14.9</td>
<td>13.8</td>
<td>16.1</td>
<td>1.13</td>
<td>0.05</td>
</tr>
<tr>
<td>22:5n–3 DPA</td>
<td>22.7</td>
<td>23.5</td>
<td>19.5</td>
<td>19.5</td>
<td>1.66</td>
<td>0.05</td>
</tr>
<tr>
<td>22:6n–3 DHA</td>
<td>1.89</td>
<td>1.80</td>
<td>1.53</td>
<td>1.56</td>
<td>0.272</td>
<td>NS</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>4685</td>
<td>4976</td>
<td>4880</td>
<td>4895</td>
<td>737</td>
<td>NS</td>
</tr>
<tr>
<td>n–6:n–3 ratio</td>
<td>2.27</td>
<td>2.02</td>
<td>2.00</td>
<td>1.88</td>
<td>0.055</td>
<td>0.001</td>
</tr>
<tr>
<td>P:S ratio</td>
<td>0.07</td>
<td>0.177</td>
<td>0.199</td>
<td>0.218</td>
<td>0.0179</td>
<td>0.001</td>
</tr>
</tbody>
</table>
extent of biohydrogenation of dietary PUFA from a range of different feed types, including forages, is high, averaging approximately 86% and 92% for 18:2n−6 and 18:3n−3, respectively (Scollan et al. 2001b, 2003b). For forages, the exception is red clover. We have noted higher levels of PUFA in meat (Scollan et al. 2002) and milk (Dewhurst et al. 2003) from animals fed on red-clover based diets, which are associated with a lower degree of biohydrogenation (Lee et al. 2003). Red clover contains the enzyme, polyphenol oxidase (PPO), which is activated when red clover tissue is damaged, reducing the extent of lipolysis (Lee et al. 2004).

Understanding and developing methods of altering lipolysis and biohydrogenation of dietary PUFA in the rumen is essential to provide new opportunities for enhancing the fatty acid composition of beef and other ruminant products. Achieving this requires knowledge of these processes and of the rumen micro-organisms involved. Evidence so far suggests that the desaturation pathway is carried out almost exclusively by rumen bacteria. Nevertheless, biohydrogenation was observed by Wright (1959 and 1960) in both bacterial and protozoal fractions of rumen contents. Dawson and Kemp (1969) questioned the involvement of protoza because of their associated bacteria. They measured rates of biohydrogenation in faunated and defaunated sheep and found no differences, and thus concluded that protoza are not essential for biohydrogenation. Recent experiments carried out at the Rowett Research Institute (Wallace et al., personal communication) showed that the protoza have much higher CLA concentrations than bacteria. The exact reasons for this are currently under investigation. To date, there is no evidence that the rumen fungi play a substantial role in biohydrogenation.

The desaturation pathways require initial hydrolysis of ingested plant triacylglycerides by the plant’s own lipases (Lee et al. 2004) and by microbial lipases (Harfoot 1978), causing the release of constituent fatty acids. Anaerovibrio lipolytica is a well-known rumen lipolytic bacterium (Hungate 1966); its lipase is extracellular and has the capacity to hydrolyse diglycerides more readily than triglycerides. The lipase does not attack phospholipids and galactolipids. Bacteria that are morphologically similar to the genus, Butyrivibrio, and have the capacity to readily hydrolyse triglycerides have also been isolated (Latham et al. 1972). Phospholipase activity has also been assigned to Butyrivibrio fibrosolvens and a Butyrivibrio strain named LM8/1B (Hazelwood and Dawson 1975). Ciliated rumen protozoa have also been implicated in the hydrolysis of dietary lipid, but the presence of associated bacteria makes these observations less convincing (Hobson and Stewart 1997). There is no substantial evidence linking the fungi to hydrolysis of dietary lipid in the rumen. Regardless, before biohydrogenation of released PUFA proceeds, the fatty acid must be in the form of an unesterified fatty acid, i.e., it must have a free carboxyl group.

Because the released PUFA are adsorbed onto the surface of plant matter through hydrophobic interactions (Lough 1970), it is believed that surface-associated micro-organisms are mainly responsible for biohydrogenation (Harfoot et al. 1973). The first stage in the desaturation of both 18:2n−6 and 18:2n−3 involves an isomerisation that converts the cis–12 double bond to a trans–11 isomer, resulting in CLA and CALA (conjugated linolenic acid) respectively (Figures 2 and 3). A microbial reductase then hydrogenates the cis–9 bond with the formation of TVA.

### Table 3

Effect of concentrate vs. grass silage feeding on fatty acid composition (proportion × 100) of the phospholipid fraction in Longissimus dorsi muscle (Warren et al. 2002).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Concentrate</th>
<th>Silage</th>
<th>SED</th>
<th>$P &lt;$</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.2</td>
<td>0.3</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>16:0</td>
<td>14.8</td>
<td>15.4</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>16:1</td>
<td>0.7</td>
<td>1.5</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>18:0</td>
<td>11.2</td>
<td>10.6</td>
<td>0.12</td>
<td>0.001</td>
</tr>
<tr>
<td>18:1n−9</td>
<td>14.3</td>
<td>23.4</td>
<td>0.47</td>
<td>0.001</td>
</tr>
<tr>
<td>18:1 TVA</td>
<td>0.7</td>
<td>0.2</td>
<td>0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>18:2n−6</td>
<td>23.3</td>
<td>8.7</td>
<td>0.36</td>
<td>0.001</td>
</tr>
<tr>
<td>18:3n−3</td>
<td>0.8</td>
<td>3.7</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>cis–9, trans–11 CLA</td>
<td>0.2</td>
<td>0.1</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>20:3n−6</td>
<td>2.7</td>
<td>1.2</td>
<td>0.06</td>
<td>0.001</td>
</tr>
<tr>
<td>20:4n−6</td>
<td>10.5</td>
<td>6.3</td>
<td>0.17</td>
<td>0.001</td>
</tr>
<tr>
<td>20:5n−3 EPA</td>
<td>0.8</td>
<td>3.4</td>
<td>0.06</td>
<td>0.001</td>
</tr>
<tr>
<td>22:5n−3 DPA</td>
<td>2.1</td>
<td>4.6</td>
<td>0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>22:6n−3 DHA</td>
<td>0.2</td>
<td>0.9</td>
<td>0.04</td>
<td>0.001</td>
</tr>
</tbody>
</table>
The final step in the ruminal biohydrogenation pathways is a further hydrogenation of the trans–11 double bond, producing 18:0 (18:2n–6 and 18:3n–3 pathways; Figures 2 and 3) or 18:1 trans–15 (18:3n–3 pathway; Figure 3) (Hobson and Stewart 1997).

Kemp and Lander (1984) grouped the bacteria involved in the biohydrogenation pathways into Groups A and B. Group A bacteria were distinguished by their ability to hydrogenate PUFA to TVA, whereas Group B bacteria were distinguished by their ability to hydrogenate TVA to 18:0 (Figures 2 and 3). The main Group A bacterium is believed to be \textit{B. fibrosolvens} (Hobson and Stewart 1997), whereas the main Group B organism identified to date is the ‘Fusocillus’ sp. (Kemp et al. 1975). The latter is a genus that no longer exists in modern taxonomy, and stored isolates of this organism are no longer viable. Nonetheless, recent attempts have been made, with some success, to re-isolate this ‘Fusocillus’ or any bacteria capable of biohydrogenating TVA to 18:0. Recently, a rumen bacterium, which was classified using 16S rDNA sequencing as a strain of \textit{B. hungatei} Su6 was shown to have the ability to biohydrogenate TVA to 18:0 (van de Vossenberg and Joblin 2003). Wallace et al. (personal communication) have also re-isolated a bacterium from rumen contents which also has the capacity to transform TVA to 18:0. Using 16S rDNA sequencing, they found that the bacterium groups closely with strains of \textit{Clostridium proteoclasticum} and microscopy has revealed that it is morphologically similar to the original ‘Fusocillus’ strain. Hudson et al. (1998) have also demonstrated that the rumen bacterium, \textit{Streptococcus bovis}, has the capacity to hydrate 18:2n–6 to 13–hydroxy–9 octadecenoic acid, thus diverting it away from the biohydrogenation pathway (Figure 2). More recently, Hudson et al. (2000) discovered that other facultative anaerobic rumen bacteria, namely \textit{Staphylooccus, Enterococcus, Lactobacillus} and \textit{Pediococcus}, also have the capacity to hydrate linoleic acid. Furthermore, it has been demonstrated that a concomitant rise in \textit{Megasphaera elsdenii} occurs within the rumen following concentrate feeding (Counotte et al. 1981). \textit{M. elsdenii} causes the biohydrogenation of linoleic acid to the trans–10, cis–12 isomer of CLA (Kim et al. 2002), which explains the increases in this isomer following concentrate feeding (Figure 2). There may be many more bacteria involved in the biohydrogenation pathways, but information is limited because isolating such organisms is time-consuming. This is because biohydrogenators cannot be isolated by using specific, selective media. A wide range of culturable bacteria must first be isolated from the rumen, and then pure cultures must be screened for their ability to biohydrogenate unsaturated fatty acids. Kemp et al. (1975), for example, screened 200 isolates in order to obtain five strains that had biohydrogenation capabilities.

Proposed reasons for the occurrence of biohydrogenation have included the argument that PUFA are more toxic than saturated fatty acids to the Group A and B bacteria, requiring their desaturation within the rumen (Henderson 1973). Many more
hypotheses are arising. Notwithstanding, work is proceeding to identify many more microbial species that may be involved in biohydrogenation and to determine how they are influenced by diet. The results will lead to methods that can be used to manipulate the biohydrogenation pathways to improve the nutritional properties of ruminant products.

**Beef fatty acids and meat quality**

Increasing PUFA in ruminant tissues increases the susceptibility to oxidative breakdown of muscle lipid during conditioning and retail display. This oxidation changes flavour and promotes muscle pigment oxidation, which reduces shelf life (reviewed by Wood et al. 2003). The extent of lipid oxidation is limited by antioxidants in tissues, which include vitamin E (either added to the diet or present naturally) and other phenolic compounds from the diet.

Steers fed a concentrate diet containing fish oil had significantly higher levels of TBARS (thiobarbituric acid–reacting substances, a measure of lipid oxidation) than those fed concentrates containing Megalac or linseed at 4, 8 and 11 days of simulated retail display (Vatansever et al. 2000). Their meat also showed greater colour deterioration and the level of vitamin E in muscle of animals fed fish oil was also lower than that of those fed Megalac because of its greater utilisation in the more unsaturated membrane lipids. Associated taste panel studies gave lower scores for overall liking of meat from animals fed diets containing fish oil. Feeding linseed, which resulted in a linolenic acid content of 1.2% of total lipids in the meat, had no negative effects on quality characteristics. However, when the content of linolenic acid in the meat was increased to 2.8% of total lipid using ruminally protected lipid, sensory scores for ‘abnormal’ and ‘rancid’ were recorded (Scollan et al. 2004). These results support the conclusion by Wood et al. (2003) that only when concentrations of linolenic acid approach 3% of lipids are there any adverse effects on lipid stability, colour stability or flavour.

Beef from pasture–fed animals does have higher concentrations of more oxidisable \( \alpha \)-linolenic acid in muscle lipids, but the meat is more resistant to lipid oxidation than that from grain–fed cattle. Warren et al. (2002) compared beef from Holstein–Friesian and Aberdeen Angus steers given diets based on grass silage or concentrate. TBA values were four–fold and six–fold higher in steaks from concentrate–fed animals after 4 d and 7 d of retail display, respectively (Figure 4a). Steaks from silage–fed animals had a retail colour shelf–life that was 5 d longer than that of steaks from concentrate–fed animals at 11 d and 6 d (Figure 4b). These effects were related to higher levels of vitamin E in the meat from animals fed grass–silage. Gatellier et al. (2004) also noted increased activity of some anti–oxidant enzymes in animals fed pasture. However, there is some evidence that the benefits of pasture feeding may not apply for meat that is processed further by mincing (Realini et al. 2004).

The differences in the fatty acid composition of meat induced by feeding grass rather than concentrates have been reported to affect beef flavour. Larick and

![Figure 3](image-url)
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Turner (1990) showed that the scores for flavour descriptors changed when cattle that were previously grass–fed were changed to a maize diet in a feedlot. As the period of maize feeding increased, the concentration of 18:3\textsubscript{n–3} in muscle phospholipid declined and that of 18:2\textsubscript{n–6} increased. Flavours identified as ‘sweet’ and ‘gamey’ declined, whereas ‘sour’, ‘blood like’ and ‘cooked beef fat’ increased. Flavour chemists have demonstrated that lipid breakdown products such as aldehydes and ketones are involved in these flavour differences (Larick and Turner 1987; Elmore et al. 1997). However, taste panellists in Ireland (French et al. 2000b) and in Canada (McCaughey and Ciplet 1996) found no difference in the flavour of grass vs. grain–fed beef. The authors suggested that this was due to higher antioxidant concentrations, which limited lipid oxidation reactions and the production of ‘off–flavours’ in the grass used in these studies.

However, reactions to grass or grain–fed products reflect, to some extent, the previous experience of the taste panellists (Sanudo et al. 1998). The study conducted by Warren et al. (2002) showed that feeding grass silage produced a higher beef flavour intensity in loin steaks as identified by the trained taste panel. There was also lower abnormal flavour intensity and this was apparent in topside roasts and minced beef. Meat from grass silage–fed animals had a slightly higher ‘livery’ note, which seems characteristic of grass feeding.

There is some evidence that feeding beef cattle red clover diets can reduce colour shelf life (Scollan et al. unpublished). It has been reported that milk from cows fed red clover contained more 18:2\textsubscript{n–6} and 18:3\textsubscript{n–3} PUFA than that of those fed grass silage, 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 red clover was negatively affected (Bertilison and Murphy 2003).

**Conclusions**

Evidence of interactions between nutrition and a number of lifestyle diseases (particularly heart disease and cancer) has focused attention on the role of metabolically important fatty acids. Enhancing the levels of these fatty acids in ruminant products, which are natural carriers of \(\text{n–3} \) PUFA (and CLA), would make these products more attractive to consumers and add value. Nutritional opportunities exist to produce beef characterised by a lower content of atherogenic saturated fatty acids, a higher content of more beneficial monounsaturated fatty acids and polyunsaturated fatty acids and a lower \(n–6:n–3\) PUFA ratio. It is difficult to achieve a large shift in the P:S ratio by nutritional means because of the high degree of biohydrogenation of dietary PUFA in the rumen. However, studies in which PUFA were provided either post ruminally via direct infusion into small intestine or by using rumen–protected lipids confirmed that beef muscle does have a high capacity to incorporate beneficial PUFA. Our understanding of the relationships between the fatty

![Figure 4](image-url)  
**Figure 4** Effect of breed (HF, Holstein–Friesian and AA, Aberdeen Angus) and diet on (a) mean TBA reacting substances (degree of lipid oxidation) of beef loin steaks after 4 and 7 days’ display in a modified atmosphere pack (MAP) and (b) colour shelf–life of beef loin steaks displayed in MAP (‘Conc’ = concentrate).
acid composition of meat, its sensory attributes and colour shelf life is increasing. As the concentrations of n–3 PUFA in the meat are increased, sensory attributes such as ‘greasy’ and ‘fishy’ are less noticeable, but colour shelf life may be reduced. Forage systems based on grass and clover are very important for the beef industry in many regions of the world. These systems also have the potential to produce healthier beef with improved flavour.

Consideration must be given to the target consumer base when considering ways to exploit this research. Awareness of functional foods has increased in recent years. The term, functional foods, is a generic term used to describe foods or food components that have beneficial effects for 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. ω–3 and/or CLA enriched products can make a valuable contribution in this respect and, in some countries, the dairy industry has proactively developed such products. The problem with this approach is that it will only target a limited consumer base, consisting mainly of consumers who can afford to pay for such products. However, in terms of health maintenance and disease prevention in the wider consumer base, it is more appropriate to consider application of this research to improving the healthiness of ruminant products in general.

References


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