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Enhancing the nutritional and health value of beef lipids and their relationship with meat quality

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Abstract

This paper focusses on dietary approaches to control intramuscular fat deposition to increase beneficial omega-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid content, and reduce saturated fatty acids in beef. Trans-fatty acids in beef lipids are considered, along with relationships between lipids in beef and colour shelf-life and sensory attributes. Ruminal lipolysis and biohydrogenation limit the ability to improve beef lipids. Feeding omega-3 rich forage increases linolenic acid and long-chain PUFA in beef lipids, an effect increased by ruminally-protecting lipids. Increasing beef PUFA can alter flavour characteristics and shelf-life. Antioxidants, particularly α-tocopherol, stabilise high concentrations of muscle PUFA; however, additional antioxidants are required. Currently, the concentration of long-chain omega-3 PUFA achieved in beef lipids (except animals fed ruminally-protected lipids) falls below the limit considered by some authorities to be labelled as a source of omega-3 PUFA. The mechanisms regulating fatty acid isomer distribution in bovine tissues remain unclear.Further enhancement of beef lipids requires greater understanding of ruminal biohydrogenation.

Keywords: Beef; Nutrition; Meat quality; Fatty acids; Health

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1. Introduction

The nutritional value is an important contributor to the overall quality of meat. Consumers are increasingly aware of the relationships between diet, health and well-being resulting in choices of foods which are healthier and more nutritous (Hocquette, Botreau, et al., 2012; Verbeke, et al., 2010).Intramuscular fat level, fatty acid composition, and along with the biological value of the protein, trace elements and vitamins, are key factors contributing to nutritional value(Wyness, 2013). Considerable attention has been given to enhancing beneficial fatty acids in meat and milk (Givens, 2010; Salter, 2013; Scollan, Hocquette, et al., 2006; Shingfield, Bonnet, & Scollan, 2013). Much of this research seeks to support the guidelines for fat intake by the World Health Organization (WHO;(World Health Organisation, 2003)). The WHO (2003)recommended that total fat, saturated fatty acids (SFA), *n*-6 polyunsaturated fatty acids (PUFA), *n*-3 PUFA and *trans* fatty acids should contribute < 15-30, < 10, < 5-8, < 1-2 and < 1 % of total energy intake, respectively. A recent meta-analysis of epidemiological studies has called into question the evidence that supports the association between SFA and cardiovascular disease (CVD) (Siri-Tarino, Sun, Hu, & Krauss, 2010a). Emphasis has been placed on reducing the intake of SFA (considered to be associated with increased cholesterol) and increasing the intake of omega-3 PUFA, and indeed epidemiological and clinical data support a beneficial effect of substituting SFA with PUFA, as opposed to substitution with carbohydrate (Siri-Tarino, Sun, Hu, & Krauss, 2010b). The beneficial effects of the longer chain *n*-3 PUFA, eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) in reducing the risk of cardiovascular disease, cancer and type-2 diabetes, and their critical roles for proper brain function, visual development in the foetus and for maintenance of neural and visual tissues throughout life are well recognised(Barceló-Coblijn & Murphy, 2009; Lopez-Huertas, 2010; Russo, 2009; Simopoulos, 1991).

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Intramuscular fat in muscle of mature beef consists proportionally on average of 0.45 - 0.48 as SFA, 0.35 – 0.45 monounsaturated fatty acids (MUFA) and up to 0.05 PUFA, respectively. The polyunsaturated : saturated fatty acid (P:S) ratio for beef is typically low at around 0.1 except for very lean animals (<1% intramuscular fat) where P:S ratios are much higher ~ 0.5-0.7 (Scollan, Hocquette, et al., 2006). The *n*-6:*n*-3 ratio for beef is beneficially low (usually < 3), reflecting the significant amounts of desirable *n*-3 PUFA, particularly α-linolenic acid (18:3*n*-3)but also EPA, docosapentaenoic acid (DPA; 22:5*n*-3) and DHA. Beef and other ruminant products are important dietary sources of conjugated linoleic acid (CLA) of which the most prominent is *cis*-9,*trans*-11 isomer, which has been identified to contain a range of health promoting beneficial properties(Salter, 2013). Beef lipids also contain trans-fatty acids (TFA) of which the most dominant is *trans*-11 18:1 (vaccenic acid). There is much interest in TFA produced by ruminants (rTFA) with emphasis on potential protective effect against development of coronary heart diseases, as distinct to industrial *trans* fatty acids (iTFA) (Salter, 2013; Wang, Jacome-Sosa, & Proctor, 2012).Hence considerable effort has been devoted to improving the fatty acid composition of beef.

This paper reviews recent progress in the field including the important relationships between lipids and components of meat quality such as colour shelf life and sensory attributes. Although genetics does influence intramuscular fat deposition and fatty acid composition(Hocquette, et al., 2010), this paper is focused on the nutritional influences on muscle lipids, as it is the major contributory factor(De Smet, Raes, & Demeyer, 2004). Reference is also made to recent research in vitamin and antioxidant content of beef.

2. Strategies to influence intramuscular fat deposition

Whereas intramuscular fat level is associated with juiciness, flavour, tenderness and overall liking (Jeremiah, Dugan, Aalhus, & Gibson, 2003; O'Quinn, et al., 2012), it might be

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considered as prejudicial for human health since WHO recommendationsare to reduce fat consumption(World Health Organisation, 2003). Therefore, different strategies were developed to reduce intramuscular fat level by genetic or nutritional factors.

Certain genotypes, for example, double-muscled genotypes, have been characterised by an altered metabolic and endocrine status associated with a reduced fat mass in the carcass and an orientation of muscle metabolism towards the glycolytic type (Hocquette, et al., 2010). Similarly, a high muscle growth potential induced by genetic selection is associated with a reduced fat mass in the carcass and a switch of muscle fibres towards the glycolytic type with less intramuscular fat level (Hocquette, Cassar-Malek, et al., 2012). However, from studies on differential expression of genes associated with muscle growth, it seems that genes involved in muscle mass development probably differ from those implicated in the control of fat deposition (Bernard, Cassar-Malek, Renand, & Hocquette, 2009)suggesting that the biological mechanisms governing muscle growth and fat deposition are different. Other authors consider that intramuscular fat deposition is closely linked to muscle growth since both processes are physiologically in competition for nutrient use(Pethick, Barendse, Hocquette, Thompson, & Wang, 2007; Pethick, Harper, & Oddy, 2004). Indeed, intramuscular fat is deposited at a lower rate than muscle growth during the first periods of postnatal life when average daily gain is the highest. On the other hand, intramuscular fat is deposited at a greater rate than muscle growth rate when average daily gain of animals is reduced, i.e. when animals get older. In this period (corresponding to the finishing period), intramuscular fat level inevitably increases since less nutrients are used for muscle growth (reviews from (Pethick, et al., 2007; Pethick, et al., 2004)).

Concerning the nutritional control of fat deposition, *de novo* synthesis of fatty acids in intramuscular adipocytes probably occurs mainly from glucose and less from acetate, as in other fat tissues of the carcass (reviewed by (Smith, et al., 2009)). Therefore, it has been

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hypothesized that diets that promote glucose supply to the muscle might increase intramuscular fat deposition, while limiting fat deposition in external fat tissues of the carcass. A higher glucose supply to muscles may be achieved by maximising fermentation in the rumen to produce gluconeogenic precursors (propionate) or by increasing starch digestion (releasing glucose) in the small intestine. One way to achieve this is a high level of food processing in order to maximise the accessibility of dietary starch during digestion (Rowe, Choct, & Pethick, 1999). In terms of biological mechanisms, not only may higher glucose delivery to intramuscular adipocytes be important, but also the increased levels of circulating insulin, due to a higher glucose supply which is known to stimulate lipogenesis. All these mechanisms may explain why grain feeding promotes more intramuscular fat deposition than grass finishing (reviewed by (Pethick, et al., 2004)).

3. Strategies to influence the fatty acid composition of beef

It is generally acknowledged that genetic factors have a smaller influence than dietary factors on the fatty acid composition of beef (De Smet, et al., 2004). Nevertheless, even though breed differences are generally small they do reflect differences in underlying gene expression or activities of enzymes involved in fatty acid synthesis, and therefore warrant consideration. For example, stearoyl CoA desaturase (delta-9-desaturase) mRNA expression level was related to MUFA percentage in Holstein Japanese Black cattle and a single nucleotide polymorphism (SNP) in Japanese Black cattle which contributed to higher MUFA percentage and lower melting point in intramuscular fat has been described(Taniguchi, Mannen, et al., 2004; Taniguchi, Utsugi, et al., 2004). Advances in technology and knowledge of the bovine genome have resulted in the identification of several SNP related to fatty acid metabolism in the bovine and the potential for targeted selection of animals with a particular fatty acid phenotype is increasing (for detailed discussion see (Shingfield, et al., 2013)).

As discussed by Scollan et al.(Scollan, Hocquette, et al., 2006), the content of SFA and MUFA increase faster than the content of PUFA with increasing fatness and so the relative proportion of PUFA and the P:S ratio decrease. Hence lean and late maturing breeds will have a higher P:S ratio than earlymaturing breeds when slaughtered at the same carcass weight (Raes, de Smet, & Demeyer, 2001).

The potential to alter the fatty acid composition of bovine muscle by nutrition is determined to a large extent by ruminal biohydrogenation of dietary lipids. Durand and co-workers (Durand, Scislowski, Gruffat, Chilliard, & Bauchart, 2005)demonstrated the ability to markedly increase the concentration of *n*-3 PUFA in beef muscle when 18:3*n*-3 (as linseed oil was) infused directly into the small intestine, thereby by-passing the rumen. This strategy increased the concentration of 18:3*n*-3 in total lipid from 26.3 to 176.5 mg/100 g muscle. More recently, Fortin et al. (Fortin, et al., 2010)reported that abomasal infusion of fish oil (40 g/kg dry matter intake) increased the concentration of EPA in muscle phospholipids from 4.4 in the control animals to 13.9 g/100g in the infused animals. The corresponding data for DHA were 0.69 and 3.9 g/100g. The on-going challenge is to achieve these levels of enrichment by dietary means without decreasing meat shelf-life (see below). Such changes in fatty acid composition could possibly alter flavour, but this could be an opportunity to create new markets as well as a challenge to existing markets. Subsequent to the review of Scollan et al.(Scollan, Hocquette, et al., 2006), the impact of altering the composition of the ration for cattle *per se* on the fatty acid composition of muscle has been further reviewed (Nuernberg, 2009; Palmquist, 2009; Shingfield, et al., 2013). This review will focus on very recent reports on this topic and typical responses are noted in Table 1.

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4. Forages and the fatty acid composition of beef

Forages such as grass and clover contain a high proportion (50-75%) of their total fatty acids as α-linolenic acid (Dewhurst, Shingfield, Lee, & Scollan, 2006), which is the building block of the *n*-3 series of essential fatty acids and elongation and desaturation of α-linolenic acid results in the synthesis of EPA and DHA. In temperate climates, grass, either grazed or conserved, is usually the cheapest form of cattle feed. In addition, concerns about the long term sustainability of sources of long chain *n*-3 PUFA such as fish oil in particular, have provided an impetus for examining more sustainable sources of these essential fatty acids, such as forage. In addition to the above reviews, the impact of inclusion of pasture specifically in the ration of beef cattle on the fatty acid composition of beef has been reviewed (Daley, Abbott, Doyle, Nader, & Larson, 2010; Moloney, Fievez, Martin, Nute, & Richardson, 2008; Morgan, Huws, & Scollan, 2012). The findings of the large number of studies now available are generally consistent. Thus, feeding fresh grass compared to concentrates, results in higher concentrations of *n*-3 PUFA in muscle lipids, both in the triacylglycerol and phospholipid fractions. Argentine beef was reported to contain 15 and 4 mg EPA/100g and 12 and 6 mg DHA/100g beef for pasture and feedlot beef, respectively (Garcia, et al., 2008), while beef from the United States was reported to contain 8 and 4 mg EPA/100g and 1.49 and 1.46 mg DHA/100g, for pasture and concentrate-fed steers, respectively (Leheska, et al., 2008). Feeding steers concentrates for 2 months prior to slaughter subsequent to grazing, decreased the proportion of *n*-3 PUFA (and increased the proportion of *n*-6 PUFA) in muscle (Aldai, et al., 2011). Feeding forage compared to concentrates during the finishing period is frequently associated with a decrease in the concentration of SFA and an increase in the concentration of MUFA in muscle (Shingfield, et al., 2013). This conclusion needs to be interpreted with caution given the earlier comments about the effect of fatness *per se* on the fatty acid composition of beef, and since cattle

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finished on concentrates *ad libitum* are generally fatter than similar animals finished on forage-based diets when slaughtered at the same time.

With regard to the type of forage, the fatty acid composition of muscle from cattle that grazed alfalfa, pearl millet or a mixed pasture of bluegrass, orchardgrass, tall fescue and white clover before slaughter was largely similar but the concentration of 18:3*n*-3 was highest for steers grazing alfalfa (Duckett, Neel, Lewis, Fontenot, & Clapham, 2013)(Table 1). A similar finding was reported by Moloney et al. (Moloney, McGilloway, & French, 2007)for steers grazing a white clover–rich pasture compared to a perennial ryegrass pasture before slaughter. In contrast, Dierking et al. (Dierking, Kallenbach, & Grun, 2010) observed no difference in the fatty acid composition of muscle from steers that grazed tall fescue, tall fescue/red clover-rich pasture or alfalfa before slaughter. There is increasing interest in cattle production from botanically diverse pastures but there is a paucity of information on the fatty acid composition of beef produced from such pastures. Fraser et al. (Fraser, et al., 2007) reported that inclusion of a period of grazing a *molina caerulea*(purple moor grass) dominated semi-natural pasture increased the proportion of n-3 PUFA in muscle lipids. A review by Moloney et al. (Moloney, et al., 2008) considered studies that compared grazing of a ryegrass pasture with unimproved saltmarsh pasture (Whittington, Dunn, Nute, Richardson, & Wood, 2006),grazing of ryegrass pasture with a botanically diverse pasture (Lourenco, Van Ranst, De Smet, Raes, & Fievez, 2007), grazing of a lowland pasture with a mountain pasture (Adnoy, et al., 2005), andindoor feeding of ryegrass silage with botanically diverse silage from natural, unfertilised grassland (Lourenco, De Smet, Raes, & Fievez, 2007). In this review, a general tendency for an increase in *n-3* and total PUFA proportions in intramuscular fat was observed for the botanical diverse pastures compared to the perennial ryegrass/lowland pastures. For a comprehensive review of this topic the reader is referred to Lourenço et al. (Lourenco, Van Ranst, Vlaeminck, De Smet, & Fievez, 2008).

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Table 1. Effect of forage type, oil supplementation and ruminally protected lipid supplements on the total fatty acids (mg/100g muscle) and the fatty acid composition of beef muscle (g/kg fatty acids)

	Sex	Total	14:0	16:0	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-3	20:5*n*-3	22:5*n*-3	22:6*n*-3	Reference
						Forage						
Pasture	Bulls	547	6.0	158	159	189	145.5	34.7	10.2	13.0	0.9	(Aldai, et al., 2011)
1-month concentrate after pasture	Bulls	813	10.9	184	147	202	130.5	22.1	9.2	11.5	0.7	
2-month concentrate after pasture	Bulls	1055	13.6	210	153	232	113.0	13.4	7.3	8.9	0.8	
Mixed pasture	Steers	2150	23.6	250	170	328	25.9	11.7	5.4	8.5	0.9	(Duckett, et al., 2013)
Alfalfa	Steers	2060	25.3	257	168	323	28.5	13.2	6.0	9.1	1.0	
Red clover silage	Steers	2250	24.0	256	167	339	22.7	19.6	4.9	7.6	0.7	
						Oil supplementation						
Grass silage	Steers	3179	NR	845	425	1123	47	29	17	NR	3.3	(Kim, Richardson, Gibson, & Scollan, 2011)[1]
Grass silage + echium oil (low)	Steers	4090	NR	1127	576	1378	52	31	16	NR	3.3	
Grass silage + echium oil (high)	Steers	4075	NR	1108	541	1358	54	32	15	NR	2.7	
Grass silage + linseed oil (high	Steers	3385	NR	890	474	1117	50	31	17	NR	3.4	
Hay	Steers	5680	27.5	266	113	380	26.0	5.1	2.4	4.4	NA	(Nassu, et al., 2011)
Hay + linseed	Steers	5875	27.8	234	117	347	24.0	12.2	2.7	4.0	NA	
Barley silage	Steers	6772	27.5	260	114	406	21.0	3.1	1.3	3.0	NA	
Barley silage + linseed	Steers	6413	28.0	236	119	386	21.2	10.6	2.3	3.6	NA	
						Ruminally protected oils						
Grass silage	Steers	2551	NR	665	325	880	56.7	26.9	14.3	NR	2.4	(Kim, Richardson, Lee, Gibson, & Scollan, 2010)[1]
Grass silage + plant extract (low)	Steers	2501	NR	623	332	850	70.2	38.3	16.1	NR	2.3	
Grass silage + plant extract (high)	Steers	2433	NR	596	339	794	72.7	41.3	18.3	NR	2.8	
Control	Heifers	2870[†]	86.6	730	283	937	80.4	13.3	13.0	NR	3.4	(Dunne, et al., 2011)[1]

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Protected fish oil (275 g/d)	Heifers	3890†	132	953	388	1083	82.0	27.9	52.3	NR	15.4

[1] Individual fatty acids mg/100g muscle; † intramuscular fat chemically determined; NR= not reported

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Generally levels of *n*-3 PUFA are higher in muscle from cattle fed on fresh compared to conserved grass, and increase with the amount of pasture consumed and the length of time on pasture (Scollan, Costa, et al., 2006). Replacing grass silage with a mixture of grass and red clover silage increased the deposition of *n*-3 PUFA in muscle of finishing cattle but this did not result in an increase in EPA and DHA (Lee, Evans, Nute, Richardson, & Scollan, 2009), while replacing grass silage with either whole crop wheat silage or maize silage decreased the deposition of *n*-3 PUFA in muscle from finishing cattle (Moloney, Mooney, Kerry, Stanton, & O'Kiely, 2013).

5. Supplementary lipids and the fatty acid composition of beef

5.1. Unprotected lipids

The main sources of supplementary fatty acids in ruminant rations are plant oils, oilseeds, fish oil, marine algae and fat supplements (Woods & Fearon, 2009). Since dietary inclusion of fatty acids must be restricted (to 60 g/kg dry matter consumed, approx.) to avoid impairment of rumen function, the capacity to manipulate the fatty acid composition by use of ruminally-available fatty acids is limited. Despite ruminal biohydrogenation, a proportion of dietary PUFA bypasses the rumen intact and is absorbed and deposited in body fat (Shingfield, et al., 2013). The effect of dietary supplementation with plant oils has also been recently reviewed by Ladiera and co-workers(Ladeira, Machado Neto, Chizzotti, Oliveira, & Chalfun Junior, 2012). In general, supplementation with linseed/linseed oil or flaxseed (rich in 18:3*n*-3) can increase the concentration of 18:3*n*-3 in tissue with an associated desirable decrease in the *n*-6:*n*-3 PUFA. Similarly, sunflower seed or sunflower oil (rich in 18:2*n*-6) can increase the concentration of 18:2*n*-6 in tissue but with an associated undesirable increase in the *n*-6:*n*-3 PUFA ratio. Dietary inclusion of 18:3*n*-3 generally also increases the concentration of EPA and the concentration of DHA in some (Herdmann, Martin, Nuernberg,

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Dannenberger, & Nuernberg, 2010) but not all (e.g. (Corazzin, Bovolenta, Sepulcri, & Piasentier, 2013; Juarez, et al., 2011)) studies. Kim et al. (Kim, et al., 2011)found that supplementation of grass silage with echium oil, a source of stearidonic acid (18:4*n*-3) did not increase the concentrations of EFA and DHA in bovine muscle. There seems to be little evidence of a basal forage by supplementary PUFA interaction with regard to the concentrations of *n*-3 PUFA in muscle lipids (eg. (Nassu, et al., 2011), Table 1). Supplementation with PUFA generally causes a modest but statistically significant decrease in SFA proportion and in particular the C16:0 proportion of intramuscular lipids (Moloney, 2011).

Dietary inclusion of fish oil (rich in both the long-chain *n*-3 PUFA) can increase their concentration in beef and the increase is dependent on the level of dietary inclusion (Noci, Monahan, Scollan, & Moloney, 2007; Scollan, et al., 2001). Muscle from cattle fed fish oil does not generally reach the concentrations defined by EFSA (European Food Safety Authority, 2009) to permit labelling as a "source" of *n*-3 PUFA (see final conclusion section). While the supplementation strategies described above can cause sizeable changes in the *n*-6:*n*-3 PUFA ratio, they generally do not increase the P:S ratio in the meat above the 0.1-0.15 normally observed.

5.2. Protected lipids

For practical exploitation of the capacity of muscle to deposit *n*-3 PUFA, methods to protect dietary lipids from ruminal degradation are under on-going investigation. A variety of procedures have been explored including the use of intact oilseeds, heat/chemical treatment of intact/processed oilseeds, chemical treatment of oils to form calcium soaps or amides, emulsification/encapsulation of oils with protein and subsequent chemical protection (Gulati,

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Garg, & Scott, 2005). Physical treatment methods do not greatly change the proportional loss of dietary PUFA but can increase the total amount of PUFA escaping from the rumen when cattle are fed PUFA-supplemented rations (Jenkins & Bridges, 2007). Using the latter technology above, Scollan and co-workers(Scollan, et al., 2004)showed that a protected plant oil supplement with *n*-6: *n*-3 PUFA ratio of 1:1 decreased the *n*-6: *n*-3 PUFA ratio in muscle (from 3.59 to 1.88) while maintaining the high P: S ratio. No effect was observed on the concentration of DHA. Ruminal protection of fish oil using this technology however, increased the concentration of EPA and DHA in tissue but had little effect on the P: S ratio and improved the *n*-6:*n*-3 PUFA ratio only at the highest level fed (Richardson, Hallett, et al., 2004).This may reflect the inclusion of 100 g unprotected fish oil in all treatments. Moloney et al. (Moloney, Shingfield, & Dunne, 2011)reported that long term (17 months) supplementation of beef cattle with a similar product increased the proportion of EPA and DHA in muscle phospholipids from 2.51 and 0.45 to 8.89 and 2.79 g/100g fatty acids, respectively, compared to an unsupplemented group.

While this technology seems the most effective protection strategy to date, it has not been used on a commercial scale and involves formaldehyde, the use of which may not be permitted by some regulatory authorities. Development of alternative protection technologies is continuing. Hence, fish oil encapsulated in a pH sensitive matrix which remained intact at rumen pH but which was broken down at the lower pH in the abomasum thereby releasing the fish oil for digestion, was used by Dunne et al. (Dunne, et al., 2011). This strategy also achieved a 3-fold increase in EPA but a smaller (2-fold) increase in DHA in comparison to the 3.5-fold increases observed by Richardson et al. (Richardson, Hallett, et al., 2004), probably because the fish oil contained relatively less DHA. Recent reports on the efficacy of a whey protein gel complex to ruminally protect PUFA is also encouraging in this regard (Carroll, DePeters, & Rosenberg, 2006; van Vuuren, van Wikselaar, van Riel, Klop,

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&Bastiaans, 2010). Similarly, Kronberg et al. (Kronberg, et al., 2013)reported that a supplement of flaxseed treated with a proprietary and formaldehyde-free process increased muscle 18:3*n*-3 and EPA proportion in muscle from forage-fed lambs compared to similar lambs fed a supplement of untreated flaxseed. This finding was not confirmed when the supplement was fed to cattle offered a high concentrate ration, which most likely reflects the different basal ration in both studies rather than the protection itself. Oliveira et al.(Oliveira, et al., 2012) reported an increase in 18:2*n*-6 in bovine muscle when soyabean oil was replaced by a commercial product based on calcium salts of soyabean oil. An experimental version of this process applied to linseed oil however, did not protect 18:3*n*-3from ruminal biohydrogenation(Oliveira, et al., 2012). Noci et al. (Noci, Monahan, & Moloney, 2011)reported that forming an amide derivative of camelina oil (a mixture of 18:2*n*-6and 18:3*n*-3) increased the concentration of both fatty acids in lamb muscle compared to muscle from lambs offered camelina oil but this technology has not been evaluated in cattle. Kim et al. (Kim, et al., 2010)recently reported that supplementing grass silage–fed cattle with a lipid-rich plant extract did not enhance the concentration of 18:3*n*-3, EPA and DHA in muscle (Table 1) indicating that the preparation of this extract did not result in ruminal protection.

As discussed previously, the long-chain *n*-3 PUFA are incorporated mainly into membrane phospholipids and are not incorporated into triacylglycerols to any important extent in ruminants. This provides the opportunity to manipulate intramuscular fatty acid composition of ruminant meat without large increases in fatness *per se*. Since the concentrations of EPA and DHA in fish oil are dependent on the species of fish and represent, at most, 25% of fish oil fatty acids, with the rest often being rich in SFA (Givens, et al., 2000), a prudent future strategy would be to concentrate these fatty acids prior to ruminal protection. An alternative approach is to use algae that are enriched in long-chain *n*-3 PUFA during culture. The recent report by Angulo et al. (Angulo, et al., 2012) of a marked enrichment in the proportion of DHA in muscle lipids from lactating cows supplemented with DHA-rich algae (0.06 vs. 0.3 g/100g fatty acids for a saturated fat and a linseed/DHA-algae supplemented, respectively) merit further study.

5.3. Conjugated linoleic- and trans-18:1 fatty acid isomers

Biohydrogenation of dietary PUFA by the rumen microbial system results in a broad range of intermediates being formed, such as monounsaturated *cis*- and *trans*- fatty acids (*cis*- and *trans*-18:1) and CLA isomers. Also, conjugated linolenic acids (CLnA) biosynthesized in the rumen were reported to have anti-obesity and anti-carcinogenic effects(Buccioni, Decandia, Minieri, Molle, & Cabiddu, 2012; Koba, et al., 2007). Understanding the mechanisms underlying the biosynthesis of single CLAand *trans*-18:1 isomers in the rumen is important because the ruminal outflow affects the availability of these bioactive fatty acid isomers for incorporation and *de novo* biosynthesis in different ruminant adipose tissues (Chilliard, et al., 2007; Shen, Dannenberger, Nuernberg, Nuernberg, & Zhao, 2011; Shingfield, et al., 2013). The main CLA isomer in ruminant muscle is *cis*-9,*trans*-11 CLA which accounts for more than 80% of the total CLA while*trans*-10,*cis*-12 CLA comprises 3-5% of the total CLA. However the occurrence of these bioactive fatty acid isomers is also diet dependent (Dannenberger, et al., 2005; Mapiye, et al., 2013). To date, animal and human studies have indicated that two CLA isomers, cis-9,trans-11 CLA and trans-10,cis-12 CLA, show biological activity including prevention of different types of cancer, cardiovascular health, decreasing body fat, and improved immune response(Dilzer & Park, 2012; Mitchell, Karakach, Currie, & McLeod, 2012). These effects were predominantly observed in animal models, but were inconsistent in human studies (Dilzer & Park, 2012; Mitchell, et al., 2012). There is much evidence that the physiological properties of CLA are isomer specific. Interest in the potential of CLAs to reduce chronic diseases is still in the early phase, and there are

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more questions than ever to be answered, particularly regarding mechanisms and safety concerns (Dilzer & Park, 2012).

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Table 2. Effect of diet on selected CLA isomers composition of beef muscle.

Breed	Sex	Diet	Unit	CLA isomers *tr*-11,*tr*-13	*tr*-9,*tr*-11	*tr*-11,*c*-13	*tr*-10,*c*-12	*c*-9,*tr*-11	*tr*-7,*c*-9	References
British x Continental	Steers	Barley-based, Vitamin E 340 IU	% FAME	*	*	0.01	0.012	0.25	0.084	(Mapiye, et al., 2012)
British x Continental	Steers	Barley-based, Vitamin E 690 IU	% FAME	*	*	0.01	0.013	0.25	0.093	
British x Continental	Steers	Barley-based, Vitamin E 1040 IU	% FAME	*	*	0.009	0.011	0.25	0.089	
British x Continental	Steers	Barley-based, Vitamin E 1740 IU	% FAME	*	*	0.009	0.012	0.27	0.081	
British x Continental	Steers	Red clover silage	% FAME	0.02	0.06	0.03	0.002	0.32	0.03	(Mapiye, et al., 2013)
British x Continental	Steers	Red clover silage with flaxseed	% FAME	0.10	0.22	0.33	0.002	1.41	0.08	
Limousin x Charolais	Bulls	Pasture-based, late spring	% FAME	0.030	0.024	0.038	0.007	0.361	0.013	(Pestana, Costa, Martins, et al., 2012)
Limousin x Charolais	Bulls	Pasture-based, early autumn	% FAME	0.020	0.019	0.029	0.011	0.332	0.026	
Mirandesa purebred	Calves	Barley-based, spring	% CLA	2.24	4.50	2.16	1.67	74.4	6.42	(Pestana, Costa, Alves, et al., 2012)
Mirandesa purebred	Calves	Barley-based, autumn	% CLA	1.83	4.02	1.77	1.80	74.5	7.54	
German Holstein	Bulls	Maize silage, *n*-6 concentrate	mg/100g	0.10	0.13	0.16	0.25	4.0	0.95	(Dannenberger, et al., 2013)
German Holstein	Bulls	Grass silage, *n*-3 concentrate	mg/100g	0.35	0.15	0.58	0.17	4.2	0.80	
Asturiana	Bulls	Pasture	% FAME	0.026	0.009	0.037	0.002	0.306	0.018	(Aldai, et al., 2011)
Asturiana	Bulls	Pasture, 1 month concentrate	% FAME	0.023	0.010	0.016	0.028	0.249	0.029	
Asturiana	Bulls	Pasture, 2 month concentrate	% FAME	0.017	0.010	0.022	0.019	0.302	0.038	
Asturiana	Bulls	AV *mh/mh*	% FAME	0.007	0.019	0.003	0.035	0.097	0.065	(Aldai, Dugan, Juarez, Martinez, & Osoro, 2010)
Asturiana	Bulls	AV *mh/+*	% FAME	0.007	0.011	0.005	0.034	0.178	0.079	
Asturiana	Bulls	AV +/+	% FAME	0.008	0.017	0.004	0.036	0.125	0.078	
Alentejano	Bulls	Feedlot	% CLA	1.19	2.54	1.76	0.22	81.3	8.37	(Alfaia, et al., 2009)

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Alentejano	Bulls	Pasture, finishing 4 month	% CLA	5.57	2.82	4.13	0.15	76.4	5.47	
Alentejano	Bulls	Pasture, finishing 2 month	% CLA	8.94	3.12	6.28	0.16	68.3	6.22	
Alentejano	Bulls	Pasture	% CLA	15.8	3.31	7.37	0.04	61.9	2.79	
Angus	Bulls	Natural grazing	% CLA	3.70	1.95	8.27	1.07	74.3	4.27	(Kraft, Kramer, Schoene, Chambers, & Jahreis, 2008)
Scottish Highland	Bulls	Natural grazing	% CLA	4.03	2.72	6.95	1.36	76.0	4.51	
Limousin	Bulls	Intensive production indoor	% CLA	2.38	2.40	3.67	1.94	72.8	9.93	
Simmental	Bulls	Intensive production indoor	% CLA	1.72	2.17	1.74	2.86	70.8	11.38	
German Holstein	Bulls	Maize silage, *n*-6 concentrate	mg/100g	0.1	0.2	0.2	0.2	11.7	1.4	(Dannenberger, et al., 2005)
German Holstein	Bulls	Pasture, 160 d, *n*-3 concentrate	mg/100g	0.8	0.4	2.9	0.4	14.4	1.6	
German Simmental	Bulls	Maize silage, *n*-6 concentrate	mg/100g	0.1	0.1	0.1	0.1	6.5	0.7	
German Simmental	Bulls	Pasture, 160 d, *n*-3 concentrate	mg/100g	0.2	0.1	1.0	0.2	8.0	0.5	

*Not reported

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The CLA isomer and *trans*-18:1 fatty acid concentration in beef adipose and muscle tissues may be affected by factors such as diet, species, fatness, age/weight, fat depot site, gender, breed, and season (Tables 2 and 3). Research on mechanisms of ruminal biohydrogenation of linoleic acid or linolenic acid haves focused largely on milk fat synthesis in dairy cows (Harvatine, Boisclair, & Bauman, 2009; Shingfield, et al., 2013). Investigations of the mechanisms of CLAand *trans*-18:1 fatty acid isomer formation and deposition in other adipose tissues have been only sparsely described. Strategies to increase the main CLA isomer, *cis*-9,*trans*-11 CLA, in beef adipose tissues include pasture- and grass silage-based diets with or without dietary supplements of linseed/linseed oil, rapeseed oil/cakes containing elevated levels of 18:3*n*-3, fish oil or marine algae (Table 2). CLA isomer patterns in beef muscle are affected by both diet and, if used, the type of supplement. Pasture-based diets (rich in 18:3*n*-3) with/without supplements containing linseed/rapeseed cake or oil result in higher muscle concentrations of *trans*,*trans* CLA isomers (mainly *trans*-11,*trans*-13; *trans*-12,*trans*-14;*trans*-9,*trans*-11) and *trans*-11,*cis*-13 CLA (Alfaia, et al., 2009; Dannenberger, et al., 2005). In contrast, *n*-6 PUFA-based diets (lipids rich in 18:2*n*-6 like grains, maize silage) led to higher muscle concentrations of *trans*-10,*cis*-12 CLA;*trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA (Table 2;(Shingfield, et al., 2013)). In most dietary studies, the main CLA isomer *cis*-9,*trans*-11 CLA concentration/proportion was not affected or was slightly decreased by pasture feeding with/without supplements (i.e. diets rich in 18:3*n*-3). The highest reported *cis*-9,*trans*-11CLA (including *trans*-7,*cis*-9 CLA) concentration of 134 mg/100g muscle was measured in muscle of Wagyu steers fed a high barley diet supplemented with sunflower oil (6% of dry matter) (Mir, et al., 2002).

Dietary *trans*–monoenoic fatty acids (*trans*-fat, TFA) have been given increasing attention over the last 10 years. Recent research on rTFA has revealed a protective effect against the development of coronary heart diseases, which is in contrast to the detrimental

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effects from iTFA (Gebauer, et al., 2011; Salter, 2013; Wang, et al., 2012). However, the duration and daily amount of dietary rTFA consumption required to cause significant effects on human health are still unclear (Wang, et al., 2012). Ruminant *trans*-18:1 fatty acid isomers are quantitatively the most important TFA in beef muscle. However, comparable to the CLA isomer pattern in beef, the rTFA pattern is highly dependent upon the feeding system used (Table3). It seems that there are isomer-specific effects of *trans*-18:1 fatty acid isomers for human health(Salter, 2013; Wang, et al., 2012).Elucidation of these effects require considerable analytical effort for the determination and quantification of all single *trans*-18:1 fatty acid isomers in beef. Vaccenic acid (VA, 18:1*trans*-11) is the most abundant *trans*-18:1 fatty acid isomers in beef of pasture-based fed cattle; however barley-based diets of British x Continentalcrossbred steers results in higher concentrations of 18:1*trans*-10 compared to VA and replaced VA as the major isomer in beef muscle (Mapiye, et al., 2012). Also the muscle from feedlot-fed bulls, intensive indoor-fed Limousin bulls and Normand cull cows had higher 18:1*trans*-10 compared to VA contents (Alfaia, et al., 2009; Bauchart, et al., 2010; Kraft, et al., 2008). Current knowledge suggests, 18:1*trans*-10 is one of ‘potentially negative TFA isomers’ with regard to human health (Wang, et al., 2012). Feeding forages supplemented with linseed- or sunflower oil and algae result in elevated VA level, but also higher 18:1*trans*-9 and 18:1*trans*-10 isomer contents in muscle of German Holstein cows (Angulo, et al., 2012). Pasture- and grass silage-based diets alter the *trans*-18:1 fatty acid isomer pattern and concentrations and results in a specific decrease of 18:1*trans*-6/7/8, 18:1*trans*-9 and 18:1*trans*-10, and a specific enrichment of 18:1*trans*-13/14 and 18:1*trans*-16 compared to maize silage based diets (Aldai, et al., 2011; Dannenberger, et al., 2004). However, the mechanisms of changes in *trans*-18:1 fatty acid isomer pattern and regulation of isomer distribution in beef muscle and other adipose tissues remain unclear.

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Table 3. Effect of diet on selected *trans*18:1 fatty acid isomers composition of beef muscle

				*trans*18:1 isomers						
Breed	Sex	Diet	Unit	*tr*-6/7/8	*tr*-9	*tr*-10	*tr*-11	*tr*-13/14	*tr*-16	References
British x Continental	Steers	Barley-based, Vitamin E 340 IU	% FAME	0.136	0.215	1.42	0.386	0.208	0.068	(Mapiye, et al., 2012)
British x Continental	Steers	Barley-based, Vitamin E 690 IU	% FAME	0.147	0.215	1.44	0.420	0.205	0.065	
British x Continental	Steers	Barley-based, Vitamin E 1040 IU	% FAME	0.139	0.204	1.30	0.368	0.198	0.070	
British x Continental	Steers	Barley-based, Vitamin E 1740 IU	% FAME	0.116	0.200	1.32	0.369	0.202	0.070	
British x Continental	Steers	Red clover silage	% FAME	0.10	0.19	0.20	1.11	0.37	0.18	(Mapiye, et al., 2013)
British x Continental	Steers	Red clover silage with flaxseed	% FAME	0.36	0.40	0.51	6.37	1.40	0.50	
Limousin x Charolais	Bulls	Pasture-based, late spring	% FAME	0.11	0.10	0.089	1.64	*	0.20[a]	(Pestana, Costa, Martins, et al., 2012)
Limousin x Charolais	Bulls	Pasture-based, early autumn	% FAME	0.10	0.15	0.58	1.79	*	0.18[a]	
Mirandesa purebred	Calves	Barley-based, spring	% FAME	0.11	0.16	0.14	0.65	*	*	(Pestana, Costa, Alves, et al., 2012)
Mirandesa purebred	Calves	Barley-based, autumn	% FAME	0.08	0.18	0.18	0.80	*	*	
German Holstein	Cows	Forage, protected saturated fat	% FAME	0.07	0.15	0.20	0.60	*	*	(Angulo, et al., 2012)
German Holstein	Cows	Forage, linseed oil + algae	% FAME	0.09	0.20	0.30	1.10	*	*	
German Holstein	Cows	Forage, sunflower oil + algae	% FAME	0.05	0.20	0.40	1.90	*	*	
German Holstein	Bulls	Maize silage, *n*-6 concentrate	% all isomers	3.55	7.97	19.3	37.3	15.4	5.92	(Dannenberger, et al., 2013)
German Holstein	Bulls	Grass silage, *n*-3 concentrate	% all isomers	2.80	4.50	21.8	33.9	17.1	8.46	
Asturiana	Bulls	Pasture	% FAME	0.075	0.148	0.291	2.410	0.360	0.133	(Aldai, et al., 2011)
Asturiana	Bulls	Pasture, 1 month concentrate	% FAME	0.146	0.208	2.824	1.756	0.436	0.123	
Asturiana	Bulls	Pasture, 2 month concentrate	% FAME	0.172	0.275	2.280	1.841	0.352	0.094	
Asturiana	Bulls	AV *mh/mh*	% FAME	0.350	0.357	7.311	0.508	0.535	0.055	(Aldai, et al., 2010)
Asturiana	Bulls	AV *mh/+*	% FAME	0.423	0.427	5.805	0.884	0.564	0.096	
Asturiana	Bulls	AV +/+	% FAME	0.384	0.380	7.007	0.554	0.545	0.066	
Galician Blond	Calves	Not weaned	% all isomers	3.70	6.36	9.93	45.0	17.0	5.47	(Bispo, et al., 2010)
Galician Blond	Calves	Weaned, when 5.5 month old	% all isomers	4.83	6.94	18.9	34.4	17.0	5.08	
Galician Blond	Calves	Weaned, when 2 month old	% all isomers	6.38	6.93	40.2	19.3	13.7	3.55	
Normand cull	Cow	Basal diet	% all isomers	3.70	8.50	33.7	36.1	7.40	2.90	(Bauchart, et al., 2010)
Normand cull	Cow	With extruded linseed	% all isomers	2.60	5.01	15.6	33.2	17.8	8.90	
Normand cull	Cow	With extruded flax- and rapeseed	% all isomers	3.40	6.40	41.1	25.0	11.3	3.10	

Alentejano	Bulls	Feedlot	% FAME	0.19	0.26	1.21	0.92	*	0.19[a]	(Alfaia, et al., 2009)
Alentejano	Bulls	Pasture, finishing 4 month	% FAME	0.16	0.26	0.81	1.10	*	0.23[a]	
Alentejano	Bulls	Pasture, finishing 2 month	% FAME	0.17	0.26	0.98	1.15	*	0.29[a]	
Alentejano	Bulls	Pasture	% FAME	0.12	0.15	0.20	1.35	*	0.35[a]	
Angus	Bulls	Natural grazing	% all isomers	5.27	9.12	6.52	52.6	11.6	5.49	(Kraft, et al., 2008)
Scottish Highland	Bulls	Natural grazing	% all isomers	12.5	11.5	8.85	28.1	20.2	4.99	
Limousin	Bulls	Intensive production indoor	% all isomers	7.99	10.8	23.7	15.7	22.0	5.32	
Simmental	Bulls	Intensive production indoor	% all isomers	9.51	15.9	13.3	23.8	16.7	6.79	
German Holstein	Bulls	Maize silage, *n*-6 concentrate	% all isomers	1.77	4.76	14.0	41.6	12.4	5.16	(Dannenberger, et al., 2004)
German Holstein	Bulls	Pasture, 160 d, *n*-3 concentrate	% all isomers	1.03	3.02	3.76	49.3	17.8	7.59	

*Not reported, [a] – including 18:1*c*-14

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6. Effect of diet on colour and lipid stability

When ruminants graze pasture, their muscles are more oxidative which results from a combination of two effects: i) an increase mobility at pasture and ii) a grass (vs. maize silage)-based diet (Jurie, Ortigues-Marty, Picard, Micol, & Hocquette, 2006). This orientation towards a more oxidative metabolism associated with a higher capillarity and a lower proportion of type IIB muscle fibres explains why grazed ruminants can have darker meat with a higher pigmentation (Vestergaard, Oksbjerg, & Henckel, 2000).

In addition they accumulate more *n*-3 PUFA and these PUFA are more susceptible to oxidation(Mahecha, et al., 2010). Oxidation is considered the major cause of meat quality deterioration affecting colour, flavour, and nutritional value(Li & Liu, 2012). Much interest has been focused on the protection of *n*-3 PUFA by antioxidants such as vitamin E which protects cells against attacks from reactive oxygen species(Yang, Lanari, Brewster, & Tume, 2002). Vitamin E is a fat-soluble vitamin existing in eight different isoforms with various antioxidant activities; the most active one is α-tocopherol (Daley, et al., 2010; Descalzo & Sancho, 2008), and is the most abundant fat soluble vitamin in beef adipose tissues (Table 4). So whilst pasture-fed cattle have increased n-3 PUFA, they also have increased α-tocopherol, carotenoid, and sometimes flavanoid, concentrations in their muscle compared to grain-fed cattle (Table 4). These stabilize the meat, extending colour shelf life and reducing fat oxidation during the time of retail display (Descalzo & Sancho, 2008; Gatellier, Mercier, Juin, & Renerre, 2005; Moloney, Mooney, Kerry, & Troy, 2001; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004). The γ-tocopherol and δ-tocopherol concentrations in beef muscle are only rarely reported and are present at much lower concentrations than α-tocopherol, ranging from 0.03 to 0.08 mg/kg muscle and from 0.01 to 0.04 mg/kg muscle respectively, depending on the feeding system used (Mahecha, et al., 2010; Mahecha, et al., 2009).

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Table 4. Effect of diet on fat-soluble vitamin concentration of beef muscle (mg/kg fresh muscle)

			Fat soluble vitamins					
Breed	Sex	Diet	α-tocopherol	γ-tocopherol	δ-tocopherol	Retinol (A)	ß-Carotene	References
Limousin x Charolais	Bulls	Pasture-based, late spring	5.28	*	*	*	0.10	(Pestana, Costa, Martins, et al., 2012)
Limousin x Charolais	Bulls	Pasture-based, early autumn	5.45	*	*	*	0.09	
Mirandesa purebred	Calves	Barley-based, spring	4.27	*	*	*	0.06	(Pestana, Costa, Alves, et al., 2012)
Mirandesa purebred	Calves	Barley-based, autumn	4.58	*	*	*	0.05	
Continental crossbred	Heifers	Grass silage	5.81	*	*	*	*	(Dunne, et al., 2011)
Continental crossbred	Heifers	Grass silage, RP n-3 PUFA 69g	6.95	*	*	*	*	
Continental crossbred	Heifers	Grass silage, RP n-3 PUFA 138g	5.94	*				
Continental crossbred	Heifers	Grass silage, RP n-3 PUFA 275g	7.15	*	*	*	*	
Charolais x Limousin	Heifers	Pasture	2.63	*	*	*	*	(Rohrle, et al., 2011)
Charolais x Limousin	Heifers	Pasture, grass silage	2.43	*	*	*	*	
Charolais x Limousin	Heifers	Pasture, grass silage, restricted	1.77	*	*	*	*	
Charolais x Limousin	Heifers	Concentrate	1.14	*	*	*	*	
German Holstein	Bulls	Maize silage, *n*-6 concentrate	1.20	0.08	0.01	0.13	1.10	(Mahecha, et al., 2010)
German Holstein	Bulls	Grass silage, *n*-3 concentrate	0.93	0.04	0.01	0.10	2.00	
German Simmental	Bulls	Maize/grass silage	0.94	0.04	0.02	0.03	0.17	(Mahecha, et al., 2009)
German Simmental	Bulls	Grass silage, *n*-3 concentrate	1.04	0.06	0.04	0.03	0.18	
German Simmental	Bulls	Grass silage, *n*-3 concentrate, restricted	0.93	0.03	0.03	0.03	0.16	
Friesian	Bulls	Intensive concentrate	0.75	*	*	*	*	(De la Fuente, et al., 2009)
Crossbred	Steers	Pasture	2.36	*	*	*	*	
German Simmental	Bulls	Pasture, finishing concentrate	0.72	*	*	*	*	
Hereford	Steers	Pasture, 2 years old	3.75	*	*	*	*	
Hereford	Steers	Pasture, 3 years old	4.07	*	*	*	*	
British x Indicus	Steers	Pasture	2.06	*	*	*	0.74	(Insani, et al., 2008)
British x Indicus	Steers	Pasture, grain finished	0.79	*	*	*	0.17	
Crossbred	Steers	Pasture	3.08	*	*	*	0.45	(Descalzo, et al., 2005)

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Crossbred	Steers	Pasture, 500 IU vitamin E	3.91	*	*	*	0.63
Crossbred	Steers	Grain-based	1.50	*	*	*	0.06
Crossbred	Steers	Grain-based, 500 IU vitamin E	1.76	*	*	*	0.05

*Not reported

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Li and Liu (Li & Liu, 2012)have reviewed the effect of diet and supplementation of on-farm rations with α-tocopheryl acetate on reducing the lipid and colour oxidation of ruminant meats. They point out that whilst there are many instances where colour and lipid stability appear to be linked and in particular lipid oxidation catalysing discolouration, this is not always the case. Whilst vitamin E concentration could account for up to 79% of the variation in lipid oxidation, it was only linked to 66% of the variation incolour stability (Liu, Scheller, Arp, Schaefer, & Williams, 1996). However, a muscle concentration of 3.0 – 3.5 $mg.kg^{-1}$ tissue is sufficient for optimum colour and lipid stability in loin muscle, but this varies between muscles (Liu, Lanari, & Schaefer, 1995; Liu, et al., 1996). We have observed many times that, when changing from a low oxygen packaging system (overwrapped in air, 20% oxygen) to a high oxygen (80% oxygen modified atmosphere pack), the colour shelf life and hence colour stability improves, but the rate of lipid oxidation is increased, as seen by others (O'Sullivan, et al., 2002).

Feeding grass-silage, compared to feeding concentrates increased colour stability and reduced lipid oxidation in meat from beef steers (Warren, et al., 2008), but produced few differences in flavours as tested by a trained sensory panel. Similar animals grazing grass had more of the flavour usually associated with beef meatthan the grain-fed cattle(Richardson, Nute, Wood, Scollan, & Warren, 2004). In the studies of Tansawat et al.(Tansawat, Maughan, Ward, Martini, & Cornforth, 2013), pasture feeding produced more “barney, greasy and gamey” flavour then grain-fed beef.

Meat from animals fed diets with inadequate concentrations of vitamin E for optimum colour stability can be improved by supplementing the animal diet with α-tocopherol acetate, as previously stated. Hence, Nassu et al.(Nassu, et al., 2011)fed feedlot steers on a barley-based ration with 0, 350, 700 and 1400 I.U. α-tocopherol acetate /animal/ day for 120days. The moderate to high levels of vitamin E improved retail shelf life. Interestingly, this was

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seen for 21 day aged steak meat and for minced beef but not for 6 day aged steaks. However, it should be noted that freshmeat (i.e. not aged) has a much longer retail colour shelf life than aged meat and measurements need to be continued for many more days to be able to see differences between treatments(Ledward, 1985; Nortjé & Shaw, 1989; O'keeffe & Hood, 1981; Vitale, Perez-Juan, Lloret, Arnau, & Realini, 2014).

Even when feeding forages, the concentration of vitamin E in the meat may be inadequate for maximum shelf life, even though the concentration in the diet is high. Hence, feeding cull cows either grass silage or red clover silage, produced meat with a lower concentration of vitamin E in the meat of the red clover silage-fed animals and a poorer colour shelf life and increased lipid oxidation compared to the grass silage-fed animals (Lee, et al., 2009). The increased oxidative challenge from the increased unsaturated fatty acids incorporated into the meat from the red clover silage is one explanation. In other studies, feeding beef steers increasing amounts of red clover silage in the ration produced increasing amounts of PUFA in the meat, but reduced lipid and colour stability(Scollan, Costa, et al., 2006). Supplementing a further group of animals on the 100% red clover diet with α-tocopherol acetate increased the concentration of vitamin E in their meat and hence colour and lipid stability was similar to that in the meat of the 100% ryegrass silage-fed animals (Scollan, Costa, et al., 2006). Turning cattle out to finish on grazed grass for around 100 days after a winter on red clover silage retained the increased red clover–derived n-3 PUFA concentration in the meat, but also built up the stocks of grass-derived vitamin E, resulting in colour and lipid stable meat (Scollan, Gibson, Ball, & Richardson, 2008).

Increasing the *n*-3 PUFA intake of beef animals through feeding oilseeds can improve the concentration and proportions of *n*-3PUFA in the meat (see above) but this also produces an oxidative challenge during digestion, absorption and during retail display affecting colour shelf life. This has been rectified by feeding supplementary α-tocopherol acetate with

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concentrate diets (Daly, Moloney, & Monahan, 2007; Juarez, et al., 2012; Richardson, Wood, Ball, Nute, & Scollan, 2007)and when protected fish oils were fed to grazing animals (Dunne, et al., 2011).

A dried lucerne extract rich in protein and xanthophylls, calledPX, fed as a supplement to finishing beef steers improved both the *n*-3 and *n*-6 PUFA content of their meat (Kim, Scollan, Richardson, Gibson, & Coulmier, 2009). The PX fed alongside grass silage or concentrates also increased PUFA concentrations but also led to increased lipid oxidation in the meat during extended retail display (measured after 10 days in MAP) except in the meat from a group of animals fed the concentrate diet supplemented with PX and additional α -tocopherol acetate(Kim, et al., 2010).

Attention has moved to looking for alternative antioxidant sources to vitamin E. Gobert et al.(Gobert, et al., 2010) have reported the use of a polyphenol-rich extract for stabilising meat. A combined extract of rosemary, grape residues, citrus waste and marigold, a plant extract rich in polyphenols (PERP) was fed to cull cows on a basal ration of concentrates and straw supplemented with extruded linseed and rapeseed. A control group was compared with one fed vitamin E and one fed vitamin E and PERP. The combination of PERP and vitamin E gave more lipid stable meat than the vitamin E alone, although at 155mg/kg diet the α-tocopherol acetate was not at the optimal concentration to give the most stable meat. Colour shelf life was not measured.

Feeding different diets to beef animals, supplementing them with oilseeds or protected unsaturated lipids can both improve fatty acid composition and place an oxidative stress on the meat produced. The judicious use of antioxidants can overcome these problems.

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7. Conclusions

Nutritional quality is an increasingly important factor contributing to meat product quality. Increasing the content of n-3 PUFA and CLA (in particular cis-9, trans-11 CLA) and reducing SFA are important targets, along with increased understanding of the role of ruminant trans-fatty acids in the human diet. Nutrition is the major factor influencing the fatty acid composition of beef while both nutrition and genetics influence level of fat. Feeding n-3 rich diets such as grass and concentrates containing linseed/linseed oil, fish oil or marine algae results in beneficial responses in the content of n-3 PUFA, SFA andCLA (cis-9, trans-11 CLA)in beef lipids. Processes of lipolysis and biohydrogenation of dietary lipid in the rumen play a large role in our ability to further enhance beneficial fatty acids in beef and in this regard strategies to control or protect dietary lipids from biohydrogenation are required.

A number of countries including European Union now have guidelines on the levels of long chain PUFA that a product must contain in order for it to be labelled as 'a source of' or 'high in' *n*-3 PUFA. The European Food Safety Authority recently published the concentration of long chain PUFA that a product must contain in order for it to be labelled as 'a source of' or 'high in' *n*-3 PUFA(European Food Safety Authority, 2009). They advised a daily requirement for 250mg of EPA plus DHA or 2g of 18:3*n*-3; therefore would require a food product to contain 40 or 80mg EPA plus DHA per 100g to be labelled as 'a source of' or 'high in' *n*-3 PUFA respectively (European Food Safety Authority, 2009). Based on the studies presented in Table 1 and using 100 g/day as an appropriate figure for daily beef consumption (Scollan, Hocquette, et al., 2006)then the beef from the forage-based studies summarised in Table 1 may provide up to 17 mg/d EPA and 3.3 mg/d DHA. Some studies summarised by Scollan et al. (Scollan, Hocquette, et al., 2006)did report higher values but still less than required 40 mg/100 mg. For comparison, Dunne et al.(Dunne, et al., 2011)when

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feeding heifers ruminally protected fish oil supplement compared to a control achieved levels of 52.3 *v*. 13.0 and 15.4 *v*. 3.4, for EPA and DHA, respectively. Hence, the maximal levels of EPA + DHA delivered in beef from the studies reported would be ~ 67 mg/100 g muscle (Dunne, et al., 2011). This is also higher than the ~ 15% of the daily recommended intake for long chain PUFA and as such this beef may be noted as a "source" of long chain PUFA.Similarly, all the treatments fall below the level of 2g 18:3*n*-3 per 100 g product. These aspects present considerable challenges to approach levels of PUFA for which claims may be made.

The relationships between the fatty acid composition of meat and other chemical components including amino acids and carbohydrates and the colour shelf life and sensory properties of beef are well developed. Increasing the content of long chain n-3 PUFA reduces colour shelf life and results in sensory attributes such as fishy and greasy scoring higher. Antioxidants and in particular vitamin E, which is high in pasture fed beef, help to ameliorate the negative effects of long chain PUFA on meat quality. However, alternative sources of antioxidants are required, in addition, to vitamin E, to improve colour shelf life.

This field of research has advanced much in the last 10 years and further knowledge will augment strategies for industry to take forward resulting in improvements to the nutritional properties of beef.

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