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Case Study

ENHANCING THE FUNCTIONAL VALUE OF BUFFALO MEAT THROUGH DIETARY MANIPULATION

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ABSTRACT
The modern desire of the health conscious people is functional foods. India is the largest exporter of buffalo meat, it exports 2.16 million tonnes buffalo meat per year. India contributes 2.42% (6.5 million tonnes) of world total meat production (169 million tonnes) (FAO, 2005). Consumers are becoming more aware of the relationships between diet and health and this has increased consumer interest in the nutritional value of foods. This is impacting on the demand for foods which contain functional components that play important roles in health maintenance and disease prevention. Peoples are more aware of the relationship between the diet and health, particularly in relation to cancer, atherosclerosis, obesity and type-2 diabetes. Despite the high levels of ruminal biohydrogenation nutrition is the major route for increasing the content of beneficial fatty acids in beef. Feeding grass or concentrates containing linseed in the diet increases the content of n- 3 PUFA and its longer chain derivative eicosapentaenoic acid (EPA, 20:5n - 3) in beef muscle and adipose tissue, resulting in a lower n - 6:n - 3 ratio(Williamson *et al*., 2005). Grass feeding also increases docasahexaenoic acid (DHA, 22:6n- 3). Feeding PUFA rich lipids which are protected from ruminal biohydrogenation result in further enhancement of the PUFA in meat with concomitant beneficial improvements in the ratio of polyunsaturated: saturated fatty acids (P: S ratio) and n - 6:n -3 ratio(Scollan *et al.,* 2004). The content of conjugated linoleic acid (CLA) in milk and meat of ruminants is affected by the diet, especially by the content of polyunsaturated fatty acids in it (Enser *et al*., 1999).Therefore, it is possible to increase the content of CLA in meat from ruminant animals through the feeding diets with polyunsaturated fatty acid-rich diet. However, grass feeding not only increases n- 3 PUFA and CLA but, due to its high content of vitamin E, colour shelf life is improved.

KEYWORDS: PUFA, CLA, Biohydrogenation, Functional value.

INTRODUCTION

The modern desire of the health conscious people is functional foods. India is the largest exporter of buffalo meat, it exports 2.16 million tonnes buffalo meat per year. India contributes 2.42% (6.5 million tonnes) of world total meat production (169 million tonnes). India is fifth largest producer of meat in the world. Consumers are becoming more aware of relationships between diet and health and this has increased consumer interest in the nutritional values of foods. This increased the demand of foods which contain functional components that play important roles in health maintenance and disease prevention. Peoples are more aware of the relationship between the diet and health, particularly in relation to cancer, atherosclerosis, obesity and type two diabetes. Beef is considered to be a highly nutritious and valued food. The importance of meat as a source of high biological value protein and micro nutrients (including Vit.A.,B_6,B_{12},D,E,Fe,Zn,Se) is well recognized (Biesalski, 2005). However, over the last 10-15 years, these positive attributes have often been overshadowed due to the prominence given to several negative attributes, which include the perception that beef contains high level of fat which is rich in saturated fat, association between red meat and cancer, non nutritional issues such as scares. The relationships between dietary fat and incidence of lifestyle diseases, particularly coronary heart diseases are well established and this has contributed towards the development of specific guidelines from the WHO in relation to fat in diet (WHO, 2003).The nutrition of animals or rather the dietary fatty acid composition has a great impact on the fatty acid composition of animal fats. By using specially adjusted animal nutrition it is possible to considerably increase the proportion of ω-3 fatty acids in fats of various foods of animal origin as well as in the meat. In monogastric animals it is to change the fatty acid composition of animal fats (fats in the fat tissues as well as in the muscle tissues, and also egg lipids) to a large extent by changing the quality of dietary fats. In ruminants, on the other hand, the effect of dietary fats, due to microbiological hydrogenation of double bonds of fatty acids in the rumen, is less evident. Investigations show that in chicken meat, following the addition of ω-3 fatty acids in feed, the weight proportion of ω-3 fatty acids of the total of fatty acids increases by 5 times (from 1.6 to 8.4 %), whereas long-chain fatty acids up to even 9 times (from 0.79 to 6.83 %).

The content of conjugated linoleic acid (CLA) in milk and meat of ruminants is affected by the diet, especially by the content of polyunsaturated fatty acids in it (Enser et al., 1999) and conditions in the rumen. Dietary CLA intake in our diet is practically completely dependent on the intake of fats of ruminants, especially on the intake of milk fats and meat.

Importance of Diet in producing Functional food

Genetic selection and increased understanding of nutrition has led to tremendous improvements in the efficiency of animal production and in carcass composition and quality, at least with respect to carcass fatness and muscle yield. In general, the mode of action of these metabolic modifiers is to increase protein and muscle deposition while often simultaneously reducing fat deposition. Ruminant fat has a higher SFA and a lower polyunsaturated: saturated fatty acid (PUFA: SFA) ratio than non-ruminant fat, due to hydrogenation of dietary unsaturated fatty acids in the rumen. However, the nutritional background of meat-producing animals may alter the fatty acid composition of ruminant tissue fat.

Previous research has shown that including grass in the diet of dairy and beef cattle increased CLA concentration in milk and beef intramuscular fat, respectively. Generally, ruminant meat has greater concentration of CLA than that from non-ruminants. CLA can be naturally synthesized in the rumen of ruminant animals by bacteria Butyrivibrio fibrisolvens via the Δ-9-desaturase of trans 11 octadecanoic acid pathway (Pollard, Gunstone, James, & Morris, 1980). Therefore, it is possible to increase the content of CLA in meat from ruminant animals through the feeding diets with polyunsaturated fatty acid-rich diet. Dietary supplementation with vegetable oils including linseed oil and rapeseed oil could also increase ω3 fatty acid content in the form of linolenic acid, which could be used to synthesize long chain ω3 PUFA.

The level of CLA in beef is related with two factors –

1. The amount of CLA produced in the rumen
2. Synthesis in the tissue by delta-9 desaturase , fron ruminally produced trans vaccenic acid (TVA).

Feding PUFA rich diets increases the content of CLA in beef. Under these situations, the high level of Vitamin E is necessary to stabilize the effect of incorporating the long chain PUFA into meat. However, grass feeding not only increas the PUFA and CLA but due to its high Vitamin E content, colour shelf life is improved. It is evident that opportunities exist to enhance the content of health promoting fatty acids, CLA and Vitamin E in beef and beef products.

Nutrient composition of red meat

Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life. It also contains a range of fats, including essential omega-3 polyunsaturated fatty acids. While the nutritional composition will vary somewhat according to breed, feeding regimen, season and meat cut.

In general lean red meat is a particularly good source of protein, niacin, vitamine B6, vitamin B12, phosphorus, zinc and iron. It also provides riboflavin, pantothenic acid, and selenium.

1. Protein and amino acids

Raw red muscle meat contains around 20-25g protein/100g. Cooked red meat contains 28- 36g/100g, because the water content decreases and nutrients become more concentrated during cooking. The protein is highly digestible, around 94% compared to the digestibility of 78% in beans and 86% in whole wheat. Protein from meat provides all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) and has no limiting amino acids. The amino acid glutamic acid/glutamine is present in meat in the highest amounts (16.5%), followed by arginine, alanine, and aspartic acid.

2. Fat

There is a wide variation in the amount of total separable fat between the different beef cuts .Meat after trimming external fat, and the most recent nutritional analyses show that all trimmed lean red meats are relatively low in fat (<7%) and have moderate cholesterol content, with the exception of mince meats

3. Fatty acids

Much of the discussion about the fat content of red meat focuses on the saturated fat content. However, the amount of saturated fat in beef is actually less than the total amount of unsaturated fats on a per edible portion basis. Saturated fatty acids comprise, on average, 40% of total fatty acids in the lean component and 48% in the fat component of red meat. In beef and veal, approximately half of the saturated fatty acid in both the lean and fat component of red meat is palmitic acid (16:0) and about a one third is stearic acid (18:0).

Polyunsaturated fatty acids (PUFA) range from 11% to 29% of total fatty acids. Pasture fed beef is better source of omega-3 fats than grain feed beef, and this explains the better fatty acid ratio in red meat. Beef also have more omega-3 fatty acids than either chicken or pork.

The recent revision of the dietary intakes recommended a daily adequate intake of long chain omega-3 fatty acids (DHA, EPA and DPA) of 160mg for men and 90mg for women. Since the levels of long chain PUFA found in beef muscle meat are greater than 30mg per serving (135g) of red meat, they are considered a good source of long chain n-3 PUFA.

It is recommended that total fat and saturated fatty acid (SFA), n-6 PUFA, n-3 PUFA and trans fatty acid should contribute ,<15-30%, <10%,<5-8%, <1-2% and <1% of total energy intake, respectively. Reducing the intake of SFA (which are known to raise total and LDL of cholesterol) and increasing the intake of n-3 PUFA is particularly encouraged. Among the n-3 PUFA, eicosapentanoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) have been demonstrated to have important roles in reducing the risk of cardiovascular diseases, critical for proper brain and visual development in the foetus, the maintenance of neural and visual tissues throughout life (Leaf *et al*., 2003) and may have role in reducing cancer, obesity and type two diabetes (WHO, 2003).

Despite the high levels of ruminal biohydrogenation of dietary polyunsaturated fatty acids (PUFA), nutrition is the major route for increasing the content of beneficial fatty acids in beef. Feeding grass or concentrate containing linseed (rich in α-linolenic acid, 18:3n-3) in the diet increase the content of 18:3n-3 and its long chain derivative eicosapentaenoic acid (EPA, 20:5n -3) in beef muscle and adipose tissue, resulting in a lower n-6: n-3 ratio. Grass feeding also increases docasahexaenoic acid (DHA, 22:6n-3). Feeding PUFA rich lipids which are protected from ruminal bio-hydrogenation result in further enhancement of the PUFA in meat with concomitant beneficial improvements in the ratio of polyunsaturated: unsaturated fatty acids (P:S ratio) and n -6:n -3 ratio. The main CLA isomer in beef is CLA cis-9, trans-11 and it is mainly associated with the triacylglycerol and therefore positively correlated with level of fatness.

The level of CLA in beef is related to (1) the amount of this isomer produced in the rumen and (2) synthesis in the tissue, by delta- 9 desaturase, from ruminally produced trans vaccenic acid (18:1 trans -11; TVA). Feeding PUFA rich diets increases the content of CLA in beef. Under these situations, the high level of Vitamin E is necessary to stabilize the effect of incorporating the levels of long chain PUFA into meat. However, grass feeding not only increas PUFA and CLA but, due to its high content of Vitamin E, colour shelf life is improved. It is evident that opportunities exist to enhance the content of health promoting fatty acids, CLA and vitamin E in beef and beef products.

4.Vitamins

As with other animal foods, red meat is an excellent source of bioavailable vitamin B12, providing over two thirds of the daily requirement in a 100g serve. Up to 25% of riboflavin, niacin, vitamin B6 and pantothenic acid can also be provided by 100g of red meat. Liver is an excellent source of vitamin A and folate, but the levels in lean meat tissue are low. For all these vitamins, older animals tend to have higher concentrations.

Due to the above facts, considerable attention has been placed on improving the nutritional value of beef which is beneficial to human health and disease prevention. While protein content and amino acid profile are little influenced by animal production factor such as nutrition and genetics, it is recognised that, fat content, fatty acid composition, CLA content and vitamin E may be altered. This paper reviews strategies for increasing the content of beneficial omega-3 polyunsaturated fatty acids and conjugated linoliec acid (CLA), vitamin E and reducing saturated fatty acid (SFA) in beef.

Fatty acid profile of IMF and its relationships to human health

Intramuscular fat consists on average, of 0.45–0.48, 0.35–0.45 and up to 0.05 of total fatty acids as SFA, monounsaturated fatty acids (MUFA) and PUFA, respectively. The PUFA: SFA [P: S, taken as (18:2 + 18:3)/(14:0 + 16:0 + 18:0)] ratio for beef is typically low at around 0.1 (Choi *et al.*, 2000; Scollan *et al*., 2001), except for very lean (<1% IMF) 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, reflecting the considerable amounts of beneficial n- 3 PUFA in beef, particularly 18:3n-3 and the long chain PUFA, EPA and DHA.

The predominant SFA are 14:0 (myristic acid), 16:0 (palmitic acid) and 18:0 (stearic acid).SFA influence plasma cholesterol, though stearic acid is regarded as neutral in this regard (Yu *et al*., 1995) and palmitic acid is less potent than myristic acid (Williamson *et al*., 2005). When palmitic acid and myristic acid is consumed, they increase the concentration of low density lipoprotein (LDL) in the blood. Linoleic and α-linolenic acids are the main PUFA while oleic acid (18:1n-9) is the most prominent MUFA. The PUFA and MUFA are generally regarded as beneficial for human health. Beef also contains small amounts of the long chain C20/22 PUFA, EPA and DHA and recent research has demonstrated that red meat is an important source of these fatty acids for man (Howe *et al*., 2006).

The total IMF content generally depends on the amount of triacylglycerols, whereas the amount of phospholipid, as the building blocks of cell membranes, is relatively constant. Hence there is a strong relationship between IMF and the content of triacylglycerols which is mainly dependent on the degree of overall body fatness, breed and muscle type. In phospholipid the proportion of PUFA is much higher than in triacylglycerols, containing not only the essential fatty acids 18:2n-6 and 18:3n-3 but also their longer chain derivatives such arachidonic acid (20:4n-6), EPA, DPA and DHA.

The phospholipids play central roles in cell membrane function and the PUFA compositions strictly controlled by a complex enzymatic system responsible for the conversion of 18:2n-6 and 18:3n-3 to their longer chain derivatives (Rae *et al*., 2004). The phospholipid fatty acids are less influenced by diet, but differences in the content of n-6 and n-3 long chain PUFA do occur.

STRATEGIES FOR ENHANCING THE FATTY ACID COMPOSITION OF BEEF

Genetic and nutritional approaches have been widely studied in relation to fatty acid composition of beef, although it is reported that genetic factors generally provide smaller differences than dietary factors (De Smet *et al*., 2004). De Smet *et al.*(2004), concluded that even though breed differences are generally small they do reflect differences in underlying gene expression or enzymes involved in fatty acid synthesis. As the content of SFA and MUFA increases, faster with increasing fatness than does the content of PUFA, the relative proportion of PUFA and the P: S ratio decrease with increasing fatness. Hence the low fat content (<1%) explains the beneficially high P: S ratio (0.5–0.6). In contrast the n-6: n-3 ratio in beef is high, but may be improved by using the nutritional approaches outlined below.

A. Effect of Forages on the fatty acid composition of beef

Plants are the primary source of n-3 PUFA, both in the terrestrial and marine ecosystems. Plants have the unique ability to synthesize n-3 series of essential fatty acids and 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 amounts of these important fatty acids in fish oils.

Forages such as grass and clover contain a high proportion (50–75%) of total fatty acids as α-linolenic acid. Exploiting the potential of herbage as an alternative to marine sources of PUFA is an important nutritional strategy for enhancing the content of n-3 PUFA in beef. The transfer of 18:3n-3 from forage through meat is dependent on two important processes- i) increasing the level of 18:3n-3 in the forage (and hence into the animal) and ii) reducing the extent of ruminal biohydrogenation.

Noci *et al*. (2005) showed that both the proportion of grass in the diet and length of time on grass were important in determining the response in beef fatty acids. French *et al*.

(2000) also found significant reductions in the proportion of SFA, both 16:0 and 18:0 with grass feeding, Collectively these responses in both SFA and n-3 PUFA contribute towards beneficial changes in P:S (increasing) and n-6:n-3 ratios (decreasing).

Razminowicz *et al* (2006) noted that pasture beef relative to ‘‘conventional’’ beef or intensively produced young bulls had higher amounts of n-3 PUFA and resulted in an low n-6: n-3 ratio. Feeding steers indoors on maize silage and concentrate resulted in higher concentrations of 18:2n-6 (and less 18:3n-3) and a less favourable n-6: n-3 ratio than pasture-finished steers (Varela *et al.,* 2004). Feeding mixtures of grass and red clover relative to grass alone increased the deposition of both -6 and n-3 PUFA in muscle of finishing beef steers, resulting in increases in the P: S ratio.

TABLE 1. Influence of grass vs. Concentrate on the fatty acid composition (mg/100gm tissue) of beef

Fatty acid	Grass	Concentrate
Total	2581	1724
18:2n-6	62.0	146.9
18:3n-3	32.0	7.2
20:5n-3	17.7	4.5
22:5n-3	10.8	10.8
22:6n-3	5.0	1.3
n-6:n-3	1.2	8.9
P:S	0.09	0.24

(Warren *et al.,* 2003)

Feeding fresh grass or grass silage compared to concentrates, rich in 18:3n-3 and 18:2n-6, respectively, results in higher concentrations of n-3 PUFA in muscle lipids, both in the triacylglycerol and phospholipid fractions (Tables1; Nuernberg, Dannenberger *et al.*, -2005; Warren *et al.,* 2002). Significantly, grass compared to concentrate feeding not only increased 18:3n-3 in muscle phospholipid but also EPA, DPA and DHA (Table 2; Dannenberger *et al.*,2004; Warren *et al.*,2002). In comparison, concentrates rich in 18:2n-6 lead to higher concentrations of 18:2n-6 and associated longer chain derivatives (20:4n-6).

TABLE 2. Influence of concentrate or grass silage on fatty acid composition of the phospholipid fraction of longissimus muscle

Fatty acid	Grass silage	Concentrate
18:2n-6	8.7	23.3
18:3n-3	3.7	0.8
20:3n-6	1.2	2.7
20:4n-6	6.3	10.5
20:5n-3 EPA	3.4	0.8
22:5n-3 DPA	4.6	2.1
22:6n-3 DHA	0.9	0.2

(Warren *et al*., 2002)

French *et al.* (2000) evaluated a influence of grass feeding (g/kg DM) on fatty acid composition of beef and found the significant reductions in the proportion of SFA with grass feeding .They concluded that as the proportion of grass in feed increases the essential fatty acid in beef increases significantly (Table 3).

TABLE 3. Influence of proportion of grass (gm/kg DM in the diet) on fatty acid composition (mg/100gm) of beef longissimus muscle (g/kg DM)

Fatty acid	Grass (g/kg DM)			
	0	510	770	1000
Total	3410	4490	4020	4360
18:2n-6	120.5	105.8	94.4	85.9
18:3n-3	29.3	35.4	41.1	46.0
20:5n-3	4.9	11.0	9.8	9.4
n-6:n-3	4.15	2.86	2.47	2.33

(French *et al.,* 2000)

B. Supplementary lipids and the fatty acid composition of beef

1. Unprotected lipids

The main sources of supplementary fatty acids in ruminant rations are plant oils and oilseeds, fish oil, marine algae and fat supplements (Givens *et a*l., 2000). 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 (Wood and Enser, 1997). Thus, linseed or linseed oil (rich in 18:3n-3) can increase the concentration of 18:3n-3 in tissue with an associated desirable decrease in the n-6: n-3 PUFA ratio (Scollan *et al.,* 2001).

TABLE 4. Influence of fat sources on the fatty acid composition (mg/100gm tissue) of beef longissimus muscle

Fatty acids	Control	Linseed	Fish oil
Total	3529	4222	4292
18:2n-6	81	78	66
18:3n-3	22	43	26
20:5n-3	11	16	23
22:5n-3	15	15	16
22:6n-3	2.2	2.4	4.6
n-6:n-3	2.00	1.19	0.91

(Scollan *et al.*, 2001)

Similarly, sunflower seed or sunflower oil (rich in 18:2n-6) can increase the concentration of 18:2n-6 in tissue but with an associated undesirable increase in the n-6: n-3 PUFA ratio (; Noci *et al*., 2005). Dietary inclusion of 18:3n-3 also increases the concentration of EPA but not DHA in tissue (Scollan *et al.,* 2001). The potential of fish oil (rich in both the long chain n -3 PUFA) to increase their concentration in beef is illustrated in Table 4 (Scollan et al., 2001) and the increase is dependent on the level of dietary inclusion (Noci *et al*., 2005). 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.

2. Protected lipids

Durand *et al.,* (2005) demonstrated the potential to markedly increase the concentration of n-3 PUFA in beef muscle by infusing 18:3n-3 (as linseed oil) directly into the small intestine. Thus, infusing an amount of linseed oil similar to that consumed, increased the concentration of 18:3n-3 in total lipid from 26.3 to 176.5 mg/100 g muscle. Infusion also 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).

TABLE 5. Influence of rumen protected plant oil on fatty acid composition of (mg/100gm) of beef muscle

	Control	Protected lipid supplement(gm/day)	
		400	800
Total	4685	4976	4880
18:2n-6	120	255	279
18:3n-3	29	102	118
20:5n-3	13	15	14
22:5n-3	23	24	20
22:6n-3	1.9	1.8	1.5
n-6:n-3	2.27	2.02	2.00
P:S	0.07	0.18	0.20

(Scollan *et al*., 2004)

Various 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 *et al*., 2005). Using the later technology, Scollan *et al.,* (2003) showed that a protected plant oil supplement (with n-6: n-3 PUFA ratio of 2.4:1) markedly improved the P: S ratio (from 0.08 to 0.27) but increased the n-6: n-3 PUFA (from 2.75 to 3.59) in muscle. It is interesting that the increase in P: S was associated with an increase in PUFA content but also a reduction in IMF. 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 (Scollan *et al.,* 2004). Ruminal protection of fish oil 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. 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, often the rest being rich in SFA (Givens *et al.,* 2000), a prudent future strategy would be to concentrate these fatty acids prior to ruminal protection. Moreover, since the most effective protection strategies to date have been on a non-commercial scale and involved formaldehyde, the use of which may not be permitted by some regulatory authorities, development of alternative protection technologies is needed. The recent report on the efficacy of a whey protein gel complex to ruminally protect PUFA is encouraging in this regard (Carroll *et al*., 2006).

STRATEGIES FOR ENHANCING CLA IN BUFFALO MEAT

Conjugated linoleic acids consist of a collection of positional and geometric isomers of octadecadienoic acid.

Ruminant meats and milk and their products are the main natural source of CLA in the human diet. CLA has been linked to a multitude of potential health benefits, including inhibition of carcinogenesis, reduced rate of fat deposition, altered immune response, reduced serum lipids, antidiabetic and antiatherogenic effects (Bauman *et al*., 1999; Belury, 2003; Pariza et al., 2001). Most research has focused on two isomers: CLA cis-9, trans-11 and CLA trans-10, cis-12 (Pariza et al., 2001). Studies on pure single isomers showed that they have differences in biological activities (Banni *et al*., 2003). Dannenberger *et al*, (2004) reported 10 isomers of CLA in beef with CLA cis-9, trans-11 representing approximately 70% of total CLA isomers. Biological effects have been widely investigated for two of these isomers.The anticarcinogenic and antiatherogenic effects of cis-9, trans-11 and the anti-obesity effects of trans-10, cis-12 have been well documented.

CLA cis-9, trans-11 is the major CLA isomer in ruminant milk and meat products and is mainly deposited in the triacylglycerols (Dannenberger *et al*., 2004). CLA cis-9, trans-11 is formed during biohydrogenation of linoleic acid in the rumen and it was initially assumed that this was the source of CLA cis-9, trans-11 in milk and intramuscular fat (Harfoot & Hazelwood, 1998). However, Griinari *et al*., (2000) showed that the primary source of CLA cis-9, trans-11 is endogenous synthesis from TVA formed during ruminal biohydrogenation and involving delta 9-desaturase (Khanal & Dhiman, 2004; Song & Kennelly, 2003). Diets containing a proportionally high level of linolenic acid in the fat, such as fresh grass, grass silage, and pasture feeding during the finishing periods, resulted in increased deposition of CLA cis-9, trans-11 in muscle (Enser *et al*., 1999; French *et al*., 2003; Scollan *et al*., 2001).

De La Torre and Gruffat *et al*. (2006) have shown that not only lipid supplementation affect the proportion of CLA produced, but this also depends upon basal diet, breed, age and sex of the animals. This is particularly important because the individual CLA isomers show different biological activities (Banni et al., 2003; Pariza *et al*., 2001; Khanal, 2004). Additionally, the distribution pattern in the tissue lipids will be affected by the composition of the ration consumed (Dannenberger *et al*., 2004; De La Torre and Gruffat *et al*., 2006; Nuernberg *et al*., 2002;).

A. Pasture feeding.

A switch from concentrate-based diet to pasture has been shown to increase CLA content. French *et al*. (2003) reported a significant increase in CLA cis-9, trans-11 in muscle of crossbred steers grazed for 85 days on pasture compared to concentrate, averaging 1.08% and 0.37% of total fatty acids, respectively. Poulson *et al.,* (2001) reported a 6.6 times higher CLA content in the longissimus and semitendinosus muscle from steers raised only on forages compared to steers fed a common high grain feedlot diet (13.1 vs. 2.0 mg/g). Poulson *et al.,* (2001) reported a 6.6 times higher CLA content in the longissimus and semitendinosus muscle from steers raised only on forages compared to steers fed a common high grain feedlot diet compared to those fed only the grain based diet (8.0 vs. 2.0 mg/g). That finishing steers on pasture instead of concentrate feeding leads to higher CLA contents in intramuscular fat (5.3 vs. 2.5 mg/g in longissimus dorsi muscle) was confirmed by another study (Realini *et al.,* 2004). Grazing on pasture for about 200 days and then being shifted to a dry lot diet for about 60 days also led to significantly higher CLA concentrations in steers and heifers compared with animals offered only the dry lot diet. Contrary to these results, Nuernberg *et al.* (2005) found no significant effect of grass feeding on the CLA content in bulls and steers compared with concentrate feeding (5.6/5.2 vs. 6.0/5.5 mg/g in longissimus dorsi muscle). However, in a subsequent study Nuernberg *et al.* (2005) reported significantly higher proportions of the cis-9, trans-11 isomer in bulls and lambs after pasture feeding compared with concentrate feeding.

TABLE 6. CLA in longissimus muscle of buffalo and cattle raised on pasture

Variable	Buffalo (n=51)	Cattle (n=48)	P value
Total lipids (g/100g fresh meat)	1.60 ± 0.04	1.40 ± 0.04	0.002
C18:2 c9,c12 (mg/g of i/m lipids)	12.86 ± 0.37	13.87 ± 0.38	NS
C18:2 cis9,trans11	1.27 ± 0.04	1.01 ± 0.04	0.001
C18:2 t10,c12	0.56 ± 0.002	0.47 ± 0.02	0.003
Total CLA	1.83 ± 0.06	1.47 ± 0.06	0.0001
CLA:C18:2 c9,c12	0.10 ± 0.004	0.07 ± 0.004	0.001

(Giuffrida *et al.,* 2008)

The increased CLA content in meat from animals grazing on pasture is attributed to the high PUFA content of grass (especially n-3 18:3 with an n-6: n-3 ratio of approximately 1:3–5). Although not the only determinant, the amount of dietary PUFA determines the generation of trans fatty acids by rumen bacteria. This may be related to the reduction of sugar and soluble fibre through the ensiling process which may influence the ruminal environment of the animals consuming the silage (French *et al.*, 2000). Pasture feeding does not only cause higher CLA concentrations but also influences fatty acid composition. A decrease in the n-6:n-3 PUFA ratio as well as an increase in the PUFA: saturated fatty acids (SFA) is described in beef adipose and muscle tissue by inclusion of grass in the diet (French *et al.,* 2000; Nuernberg *et al.,* 2004; Realini *et al.,* 2004). In lambs a decrease in n-6: n-3 PUFA ratio has been documented as well (Nurrnberg *et al.,* 2001).

B. Feeding of oilseeds

Adding oilseeds to the diet has been proven to be an efficient method to increase the CLA content in the muscle lipids. However, not all oilseeds exert the same effect. Casutt *et al.* (2000) supplemented the concentrate feed to Brown Swiss bulls with sunflower, rape, or linseed oilseeds (increasing the dietary fat content by 3%). Compared to the control group (5.6 mg/g), the CLA concentration of the subcutaneous fat in the sunflower group was significantly increased (7.8 mg/g FAME, fatty acid methyl ester) whereas no changes were observed in the linseed group (5.5 mg/g FAME) and in the rapeseed group the CLA content even decreased (4.6 mg/g FAME).. The CLA content increased significantly by about 70% from 4.1 to 7.0 mg/g total fatty acids in the longissimus thoracis muscle(Silva *et al*., 2003). However, with regards to the CLA this feeding strategy was not superior to grass feeding. Two studies from Poland (Stasiniewicz *et al.*,2000; Strzetelski *et al.,* 2001) investigated the effect of adding linseed (19% of concentrate mixture) to a control diet with concentrate and whole maize plant in Black-and-White Lowland bulls. The CLA levels in the longissimus dorsi muscle increased in both studies with the linseed supplementation but only in one study to a significant extent (1.7 vs. 3.7 and 2.1 vs. 2.9 mg/g lipid, respectively). However, the CLA levels were in both studies rather low. Enser *et al*. (1999) documented markedly higher intramuscular CLA levels in the longissimus muscle of Charolais steers. By feeding them grass silage and a concentrate containing linseed instead of Megalac (a ruminally protected lipid supplement high in palmitic acid), the CLA content was significantly increased from 3.2 to 8.0 mg/g.

The fatty acid profiles and CLA in the subcutaneous fat and muscle from zebu steers fed different oilseeds are presented in Table7. The data are presented as percentages because no difference was observed in total fatty acid concentrations on a grams of tissue basis ($P = 0.85$). Oleic acid (C18:1 *cis*-9) constituted the greatest proportion in both tissues, followed by palmitic acid (C16:0) and stearic acid (C18:0). The most abundant SFA in the subcutaneous fat were palmitic, stearic, and myristic acids. Among the SFA, palmitic and myristic acids attract the most attention because they are considered hypercholesterolemic. When palmitic and myristic acids are consumed, they increase the concentrations of low-density lipoproteins (LDL) in human blood, making reduction of their concentrations potentially beneficial. According to Woollett et al. (1992), these acids interfere with the normal operation of the LDL receptors in the liver, increasing LDL concentration in the plasma.

Extruded full-fat soybeans were also shown to increase the CLA content in muscle fatty acids of crossbred Angus steers (measured in lipids of rib longissimus, eye of round, and chuck tender muscles). Adding 25.6% of soybeans in the diet, but not 12.7%, significantly increased CLA concentrations (in mg/g FAME; 25.6% soybeans: 7.7, 12.7% soybeans: 6.9, and control: 6.6). Whole crushed soybeans with crushed raw flax as a supplement in low and high-forage diets for Friesian bull calves showed higher CLA contents with raw flax (2.8 vs. 4.7 and 3.3 vs. 6.3 mg/g in low and high-forage diets, respectively) in the longissimus dorsi muscle. The compositions of fatty acids in the silage and oilseeds are presented in Table 8. Soybeans had a greater percentage of linoleic acid (C18:2), whereas cotton seed had greater percentages of myristic (C14:0) and palmitic acids (C16:0). Finally, as expected,linseed was characterized by an increased content of linolenic acid (C18:3).

TABLE 8. Percentages of the principal fatty acids in the corn, corn silage, and oilseeds

Item	Silage	Corn	Soyabean	Cottonseed	Linseed
C14:0	1.93	0.1	0.59	1.06	0.78
C16:0	20.7	16.3	13.1	22.1	12.9
C18:0	14.3	2.6	7.6	5.9	9.1
C18:1,cis 9	26.1	30.9	21.1	16.5	29.4
C18:2	24.4	47.8	51.0	47.8	23.6
C18:3	4.2	2.3	6.8	0.3	19.8
SFA	38.9	15.9	21.7	29.5	24.3
UFA	59.7	84.1	77.7	69.8	75.3
MUFA	30.5	32.3	22.7	18.3	31.6
PUFA	29.2	51.8	55.0	51.5	43.7

C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 *cis*-9 = oleic acid; C18:2 = linoleic acid; C18:3 = linolenic acid; UFA = unsaturated fatty acid

In a subsequent experiment a small increase in the CLA content was found when crushed raw flax was added to a low-forage diet (4.6 vs. 5.3 mg/g FAME), but a much larger effect was observed adding raw flax to a high-forage diet (4.0 vs. 6.7 mg/g). This led to the assumption that there is a synergistic effect of high-forage concentration and dietary supplementation with PUFA.

C. Feeding of vegetable oils

Vegetable oils as equivalent to oilseeds show similar effects on CLA content. In beef cattle the addition of 3% and 6% sunflower oil to a barley based finishing diet resulted in increased CLA contents in longissimus muscle: 2.0, 2.6 and 3.5 mg/g lipid for control, 3%, and 6% sunflower oil, respectively (Mir *et al.,* 2003). A more substantial increase in the CLA concentration can be expected when sunflower oil is added to both the growing and finishing diet of beef cattle. Added to a barley and hay-based diet sunflower oil supplementation increased the CLA content in the lipids of the longissimus muscle to 12.3 compared to 2.8 mg/g FAME in the control group (Mir *et al.,* 2002). Noci *et al.* (2005) documented in their study 4.3, 6.3, and 9.1 mg CLA/g FAME in longissimus dorsi muscle lipids of heifers after supplementing the feed with 0, 55, and 110 g sunflower oil per kg of the diet for 142 days before slaughter. Rapeseed oil and whole rapeseed do not seem to have positive effects. Of three studies (two in beef cattle and one with lambs) none showed increased CLA concentrations in the longissimus dorsi muscle after supplementation with rapeseed oil (6% of DM) (Stasiniewicz *et al.,* 2000; Strzetelski *et al.,* 2001*).*

Results regarding the effect of soybean oil supplementation on CLA content are inconsistent. Supplementing a corn-based diet of Angus-Wagyu heifers with 5% (of dry matter) soybean oil had no effect on the proportion of c-9, t-11, 18:2 in muscle tissue. In a study with steers by Griswold *et al.* (2003), supplementation of 4% soybean oil to a finishing diet based on concentrate and forage (80:20) resulted in a depression of the CLA deposition in muscle tissues (2.5 vs. 3.1 mg/g) compared to the same diet without soybean oil. On the other hand, comparing 4% with 8% added soybean oil in a 60:40 concentrate: forage diet (same study) showed a numerical increase of the CLA content with the higher soybean supplementation (2.8 vs. 3.1 mg/g).

Vegetable oils influence CLA content in meat by supplying PUFA which are substrates for bacterial isomerisation or/and bio-hydrogenation in the rumen. If the lipid is resistant to ruminal isomerisation or/and bio-hydrogenation CLA cannot be produced (neither in the rumen nor endogenously) because its precursor is not available as shown by Scollan *et al.*(2003) in Charolais steers fed with a grass silage plus concentrate diet. A ruminally protected lipid supplement comprising a mixture of soybean, linseed and sunflower seed oils was compared with the lipid source Megalac. CLA concentration of neutral lipid in muscle tissue of longissimus thoracis did not differ between treatments and the CLA content of the phospholipid fraction slightly decreased when the diet with the ruminally protected lipid mixture was given. In addition to CLA content, modifications in fatty acid composition in muscle and adipose tissues of beef cattle and lambs are reported when the diet is supplemented with unsaturated fatty acids (Mir *et al.,* 2003; Stasiniewicz *et al.,* 2000; Strzetelski *et al.,* 2001).

Both oilseeds and free oils affect CLA content and fatty acid composition in the tissues in a similar manner. Free plant oils with high PUFA concentrations are normally not included in ruminant diets as high levels of dietary fat disturb the rumen environment and inhibit microbial activity (Raes *et al.,* 2004). Additionally, vegetable oils are a rather expensive dietary supplement for ruminants and are more susceptible to oxidation than seeds. Aharoni *et al.* (2005) compared soybean oil with full fat soybeans as supplements over five months in a high forage fattening diet of Friesian bull calves. Extruded full fat soybeans were about 20% more efficient than free oil in increasing the CLA concentration in intramuscular fat. The full fat soybean supplement also resulted in higher PUFA and lower SFA and monounsaturated fatty acids (MUFA) content in the intramuscular fat than supplementation with soybean oil. This may be due to a partial protection of the oils against ruminal bio-hydrogenation by roughly crushed seeds (Casutt *et al.,* 2000). Therefore, using oilseeds instead of free oils may be the preferred option.

D. Feeding of fish oils

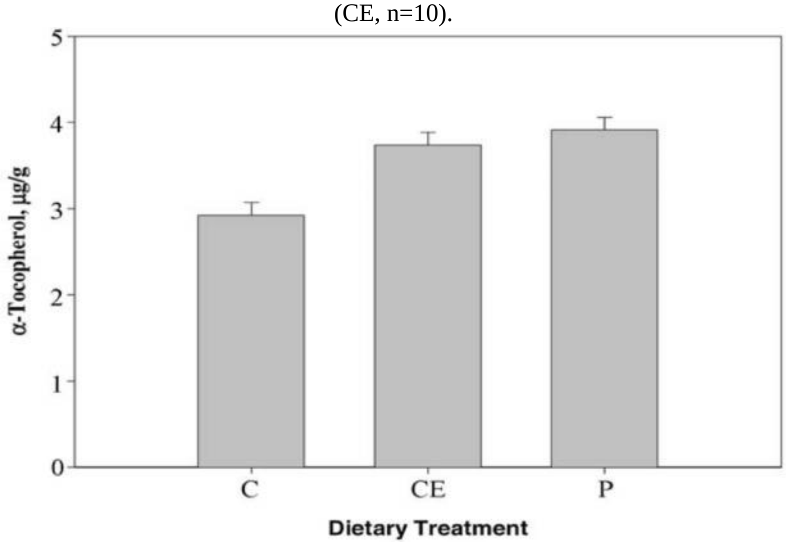
Feeding fish oil supplements is another approach to increase CLA. Enser *et al*. (1999) reported an increase in the CLA concentration from 3.2 to 5.7 mg/g in the longissimus lumborum muscles of Charolais steers fed a fish oil supplemented diet but showed simultaneously that whole linseed was more efficient in increasing the CLA concentration. The reason for the observed increased CLA levels is not clear yet, as only small amounts of linolenic and linoleic acid are present and the long chain n-3 fatty acids are not isomerised/hydrogenated to CLA or trans-vaccenic acid. Thus long chain n-3 fatty acids present in fish oil may interfere with the bio-hydrogenation of linolenic or and linoleic acids or affect delta 9-desaturase activity (Raes *et al.,* 2004). Chow *et al.* (2004) postulate that fish oil increases ruminal accumulation of trans-vaccenic acid by inhibiting the final bio-hydrogenation step to stearic acid. This would supply more substrate for endogenous CLA synthesis. However, further studies are needed to provide an explanation.

Feeding fish oil also increases the n-3 long chain PUFA concentration in the intramuscular fat due to the high eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) content in fish oil. Ruminal biohydrogenation of EPA and DHA is limited, and therefore considerable amounts of these fatty acids are available for incorporation into the adipose tissue (Raes *et al.,* 2004).

STRATEGIES FOR ENHANCING MUSCLE α-TOCOPHEROL CONCENTRATION IN BEEF

Faustman, *et al.* (1989) reported minimum tissue levels of 3.0 mg α-tocopherol/g muscle, have a significant impact on the reduction of pigment and lipid oxidation. Liu *et al.* (1996) concluded that these critical α-tocopherol concentrations might be the minimal critical levels that need to be achieved in order to enhance meat quality. Faustman *et al.* (1998) suggested that if a nutritional program delivers sufficient vitamin E to obtain the threshold level in muscle, then additional supplementation is unnecessary. α- tocopherol concentrations in fresh forage can theoretically result in muscle saturation with α-tocopherol, since green forage may be a good dietary source of α -tocopherol when pasture quality allows for high levels of α -tocopherol consumption. Yang *et al*. (2002) conducted recently in Australia showed that vitamin E supplementation of pasture-fed cattle did not alter muscle tocopherol contents.

TABLE 9. Muscle a-tocopherol concentrations from pasture (P, n=10), concentrate (C, n=10), and concentrate–vitamin E (CE, n=10).



(Faustman *et al.*, 1989)

Results from different field studies reported that 500–1000 I.U. animal/ day/ of vitamin E for 90–100 days prior to harvest is efficacious for beef marketed in both domestic and export trades (Smith *et al.*, 1996). Roeber *et al.*(2001) evaluated the effect of three supplementation levels with α-tocopherol on product shelf life, and concluded that 1000 I.U. animal/ day/ of α-tocopherol for at least 100 days can be used to increase shelf life and to improve the overall colour acceptability of steaks and ground beef products. Supplementation of α-tocopherol to concentrate-fed cattle with 1000 I.U./ animal/ day for 100 days was sufficient to achieve similar (P>0.05) muscle α-tocopherol content to grass-fed cattle, at levels beyond the proposed critical concentrations for improving shelf life. Study by cascone *et al.*, 2007 showed that supplementing vitaminE to the buffalo significantly increases the concentration of vitamine E in muscles.

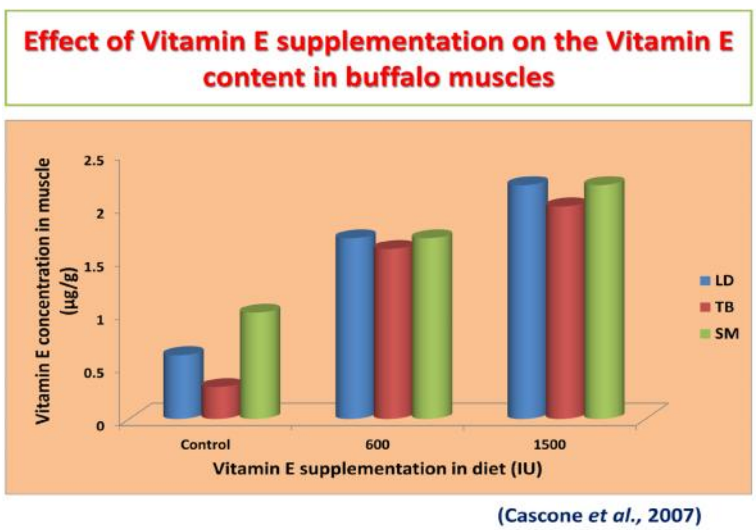


FIGURE 2. Effect of Vitamin E supplementation on the Vitamin E content in buffalo muscles

Faustman *et al.*(1998) suggested that if a nutritional program delivers sufficient vitamin E to obtain the threshold level in muscle, then additional supplementation is unnecessary. Alfa tocopherol concentrations in fresh forage can theoretically result in muscle saturation with α-tocopherol, since green forage may be a good dietary source of α-tocopherol when pasture quality allows for high levels of α-tocopherol consumption (Faustman *et al.*, 1998). Research conducted recently in Australia (Yang *et al.*, 2002) showed that vitamin E supplementation of pasture-fed cattle did not alter muscle tocopherol contents.

CONCLUSION

In conclusion, the relative proportion of CLA can be increased by grass feeding or by adding linolenic and linoleic acid rich sources as well as fish oil to high concentrate diets of beef cattle and lambs. Furthermore, the overall fatty acid profile is affected. The changes mainly depend on the supplemented fatty acids and seem to be beneficial from the human health perspective as decreased SFA and increased PUFA tissue concentrations with a lower n-6:n- 3 PUFA ratio were found.

Nutrition is the major factor influencing fatty acid composition of beef whereas both nutrition and genetics affect the level of fat. Feeding grass or concentrate containing linseed or fish oil result in important beneficial responses in the content of n-3 PUFA, SFA and CLA in beef. Studies using ruminally protected lipids have revealed that muscle does have a high capacity to deposit n-3 PUFA but strategies to address the high degree of biohydrogenation of dietary PUFA in the rumen must be developed.If a nutritional program delivers sufficient vitamin E to obtain the threshold level in muscle, then additional supplementation is unnecessary. α- tocopherol concentrations in fresh forage can theoretically result in muscle saturation with α-tocopherol, since green forage may be a good dietary source of α -tocopherol when pasture quality allows for high levels of α -tocopherol consumption.

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