PRODUCING MEAT FOR HEALTHY EATING

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Introduction

Meat is a valuable dietary source of many nutrients including a range of amino acids essential to human growth and development, fats which provide not only energy but contain essential fatty acids and their longer-chain products, minerals such as iron in a readily digestible form and some vitamins, particularly vitamin B12. Modern man developed as a hunter-gatherer eating the flesh of any animal, fish or invertebrate he could catch, along with fruits and seeds and the fossil evidence for the capture and use of wild animals is extensive. With the domestification of wild species and the development of farming, meat supply became more reliable and meat eating developed to a greater or lesser extent in different areas of the earth depending upon environmental and social factors.

Since modern man evolved as a meat eater what if anything has changed to suggest that meat may need to be altered to be a healthy component of mans diet? There are two main changes that have occurred over the last hundred years particularly in the "developed" world; one is the increase in life expectancy and the other is the availability of a relatively cheap supply of meat. With increased life expectancy came different causes of mortality, particularly coronary heart disease and cancers and causes of morbidity such as type II (maturity-onset) diabetes, autoimmune disfunction such as arthritis, and obesity have become important. The clinical investigations into coronary heart disease and its causes have been a major factor leading consumers to question the contribution of meat to a healthy diet because of its content of saturated fatty acids. Despite some detractors and some geographical variation, the cholesterol hypothesis for CHD is accepted and known to all of you. Recognition of the hypocholesterolaemic actions of the essential fatty acids linoleic (18:2 n-6) and α-linolenic (18:3 n-3) led to recommendations to increase consumption and this was fuelled by the ready availability of vegetable oils high in linoleic such as corn, sunflower and safflower. More recently the significance of the balance between these two fatty acid and their elongation and desaturation products, the n-6: n-3 ratio, was recognised as a factor not only in coronary heart disease (thrombosis) but in the immune responsiveness of tissues. These findings have led to the promulgation of dietary recommendations about fat and fatty acid consumption (DOH, 1994). The major recommendations have been to decrease fat intake as a percentage of calories to less than 35% with a mean level of 30%; to decrease the intake of saturated fatty acids to 10% or less; to decrease trans unsaturates to less than 2% and for the ratio of polyunsaturated to saturated fatty acids (P:S ratio) to be between 0.4 and 1.0. Furthermore the n-6: n-3 ratio should be less than 4. However, since the synthesis of EPA (20:5) and DHA (22:6 n-3) appears to be limited in man, even at the recommended n-6: n-3 ratio, a doubling in the consumption of preformed long-chain n-3 PUFA has been recommended from 100 mg to 200 mg/day. How meat can or might meet these requirements represents one strand of this presentation. Another is the use of meat to supply conjugated linoleic acid (CLA), a component of ruminant meat with pharmacological activity. Finally it is important that if the nutritional value of meat is improved its quality must be acceptable to consumers.

Improving the fatty acids of beef and lamb

The meat of ruminants is relatively saturated compared to non-ruminants with P:S ratios of approximately 0.1 or less (Marmer *et al.*, 1984; Enser *et al.*, 1996). However the 18:2 to 18:3 ratio is in the desirable range of less than 4 for forage fed animals but may exceed 15 in grain fed steers, reflecting the relative amounts of these two fatty acids in grain and forage respectively. Differences in tissue concentrations of linoleic and α-linolenic acid result in differences in the amounts of the longer-chain PUFA synthesized from them (Marmer *et al.*, 1984; Enser *et al.*, 1998).

Various procedures have been examined to increase the rumen bypass of dietary PUFA and to decrease the saturation of ruminant fats. Most have produced relatively small effects except those involving lipid incorporated into a formaldehyde cross-linked protein matrix (for a review see Scott and Ashes, 1993). By taking advantage of the inhibition of rumen biohydrogenation at low pH as a result of cereal feeding it proved possible to raise the P:S ratio of the *m.longissimus* in barley fed bulls to 0.3 as a result of increased deposition of linoleic acid but this caused a marked deterioration in the 18:2 to 18:3 ratio which rose to 15 (Enser, *et al.*, 1998). Although feeding larger amounts of PUFA to ruminants in an unprotected form has little effect on the P:S ratio, the small amount which escape biohydrogenation can have significant effects on tissue levels of PUFA and can meet the limited objective of doubling the concentrations of n-3 PUFA. Feeding fish meal which contains approximately 10% oil can more than double concentrations of n-3 PUFA in beef (Dawson *et al.*, 1991; Mandell *et al.*, 1997). After 168 days on a feed containing 10% fish meal, concentrations of EPA, DPA and DHA in the *m.longissimus* were (mg/100g muscle) 29.8, 15 and 10.9 compared to amounts in muscle from control steers of 5.2, 12 and 1.8 (Mandell *et al.*, 1997). Feeding fish oil directly at 3% DM doubled the concentration of EPA and DHA compared to steers fed a palm oil control (Megalac) (Table 1) (Scollan *et al.*, 1997). Mandell *et al.* (1998) reported that the meat from their steers fed fish meal which had an EPA plus DHA level greater than 25mg/100g had an unacceptable fish taint. We also detected a doubling in the scores for "fishy" by the taste panel for meat from the steers fed 3% fish oil and a slight decrease in liking (Table 1) but when fish oil was fed at 1.5% together with linseed at 1.5% oil the meat was highly acceptable (Enser *et al.*, 1997).

Although α-linolenic acid is the main fatty acid in fresh and carefully conserved forage, feeding more in the form of linseed to beef steers, 3% DM on an oil basis, doubled muscle concentrations and raised EPA by 50% compared with Megalac fed steers (Table 1). This meat was preferred by the taste panel in the UK who are used to consuming and prefer forage finished beef. Overall, these treatments achieved the desirable doubling of the concentration of n-3 and a beneficial decrease in n-6 PUFA but had no significant effects on the low P:S ratio. Trans 18:1 fatty acids were increased by the linseed and fish oil but ruminant derived trans 18:1 may have fewer harmful effects than other trans fatty acids (Willett *et al.* 1993). Alternatively the CLA present in the ruminant lipids may counteract the effects of the trans unsaturated fatty acids (see below).

Feeding n-3 fatty acid supplements to lamb as part of a complete dry feed improved the fatty acid composition of muscle lipids in a similar way to that observed in steers (Wachira *et al.* 1998). However, increases in EPA and DHA were larger than in steers and feeding linseed increased DHA deposition as well as EPA although amounts of α-linolenic were only doubled as in the steers. Overall, the concentrations of long-chain PUFA are greater in lamb than beef (Enser *et al.* 1998) which explains the differences produced by the supplements, rather than the dried grass compared with silage as the forage component of the feed. Despite the higher PUFA content of lamb, meat from those fed fish oil was as acceptable to the taste panel as that from Megalac fed lambs and, as with beef, dietary linseed increased overall liking.

Effect of breed

Koizumi *et al.* (1991) reported that EPA levels of intramuscular fatty acids were 0.35% for Japanese Yellow, 0.6% for Japanese Black and 0.98% for Hereford cattle. However, in the absence of data on total fatty acid content of the muscle it is not clear whether there was any breed effect other than on the amount of muscle lipid (marbling). We reported significant difference in absolute amounts of EPA in a beef breed, the Welsh Black, compared with the Holstein-Friesian, 18:0 versus 14.7g/100g *m.longissimus* (Choi *et al.* 1999). In sheep we have observed higher total PUFA concentrations in the small, short-tailed Soay breed than in Welsh Mountain or Suffolk x Lleyn lambs eating fresh forage. The Welsh Mountain breed also had less DPA and DHA than the Suffolk x Lleyn despite a similar concentration of α-linolenic acid (Fisher *et al.* 2000). When different breeds were fed a linseed supplement, the percentage of α-linolenic acid in the subcutaneous fat was higher for the Friesland x Lleyn and Suffolk x Lleyn lambs than in the Soays, 2.9%, 3.0% and 1.9% respectively. Linoleic acid followed a similar pattern. These results suggest that there is a limited potential to alter the synthesis and deposition of long-chain PUFA in ruminants by specific breeding programs.

Conjugated Linoleic Acids (CLA)

CLA is the name given to a range of cis trans conjugated isomers of octadecadienoic acid. CLA was identified as an anticarcinogenic compound in extracts of grilled beef (Ha, Grimm and Pariza, 1987) and has been extensively investigated by Michael Pariza and his colleagues at the University of Wisconsin, Madison. CLA occurs naturally in ruminants and ruminant products as the 9-cis, 11-trans isomer. It is formed by a bacterial isomerase in the rumen as the first stage in the biohydrogenation of linoleic acid to stearic acid (Kepler and Tove, 1967). This isomer can also be formed by stearoyl-CoA Δ9 desaturase from 11-trans octadecenoic acid (trans vaccenic acid), another product of rumen biohydrogenation (Pollard *et al.* 1980). The 9-cis, 11-trans isomer is believed to be responsible for the anticarcinogenic activity of CLA (Ha, Storkson and Pariza, 1990; Ip, Chin, Scimeca and Pariza, 1991). Epidemiological studies have suggested that high intakes of cows milk can decrease the incidence of breast cancer and this action has been attributed to the CLA in milk (Knecht *et al.*1996). The anticancer properties of CLA and milk have been reviewed by Parodi (1999). In addition to its anticancer activity CLA has antiatherogenic activity, modulates the immune system, alters the partition of energy towards protein deposition instead of fat and has antidiabetic properties in fatty (fa/fa) rats (Lee, Kritchevsky and Pariza, 1994; Cook *et al.* 1993; Park *et al.* 1997; Houseknecht *et al.* 1998). However, many of the studies have been performed using chemically synthesized mixtures of CLA and it is clear that different isomers affect different physiological systems (Lee, Pariza and Ntambi, 1998). Possible mechanisms of action of CLA include the formation of eicosanoid isomers and gene regulation. CLA is a ligand for peroxisome proliferator-activated receptors (PPAR) which are factors that control the transcription of certain genes including those that regulate the development of adipocytes (for a review see Vanden Heuvel, 1999).

In view of its potential to improve the health of mankind there have been many studies of the factors which regulate the levels of CLA in bovine milk but few investigations into meat. Since CLA production is part of the normal process of biohydrogenation of linoleic acid in the rumen, factors which inhibit this process are likely to affect CLA levels. Jiang *et al.*, (1996) demonstrated that a low forage to concentrate ratio, which decreases biohydrogenation by lowering rumen pH, increased milk CLA and that there was a strong correlation (r=0.78) between the concentrations of CLA and trans vaccenic acid (11-trans 18:1) indicating that the whole hydrogenation pathway was decreased under these conditions. However, Dhiman *et al* (1997, 1999) reported higher CLA contents in milk from grazing cows compared to those fed concentrates despite the lower linoleic acid content of the latter. The advantage of a grazing diet over concentrates in producing higher CLA levels in meat was demonstrated by Shanha *et al.*, (1997) who observed 0.74% CLA in lipids from the semitendinosus muscle of grazing steers compared to 0.51 in grazing steers supplemented with 8.5kg/day of cracked corn. The concentration in grazing animals was similar to the level we observed with linseed supplementation of a 60:40 silage:concentrate feed (Enser *et al.*, 1999)

Dhiman *et al.* (1999a) reported a doubling of the CLA in milk from cows when 12% full fat cottonseed or soybeans were added to the feed. Both soybean oil and linseed oil increased milk CLA levels 2-3 fold (Dhiman *et al.* 1997) with soybean oil being significantly more effective despite its lower level of α-linolenic acid which is a better inhibitor of biohydrogenation than linoleic acid. As expected the effects of free oil were greater than those when the oil was supplied in cracked seed. More recently we have compared the effect of feeding full fat soya and whole linseed at equal fat levels (3.5% of total dietary lipid) on the CLA content of the tissues of Charolais steers (Enser *et al.* Unpublished). After 90 days on feed levels of CLA were significantly higher in both the neutral lipid (marbling) and the phospholipids of *m.longissimus* from linseed fed steers. Although the differences within adipose tissue were similar they did not reach significance. The total muscle CLA content for steers fed the linseed diet was similar to that we previously observed for steers with a dietary forage to concentrate ratio of 60:40 (Enser *et al.*, 1999) In terms of tissue total fatty acids, the CLA levels in muscle are about half of those reported in milk.

Including 3% fish meal in the feed, which would be equivalent to 0.3% fish oil, increased milk CLA levels by 62% (Dhiman *et al.*, 1997) These results are similar to those we reported at last years 45th ICOMST (Enser *et al* 1999a, b) demonstrating that CLA concentrations in beef longissimus were doubled by feeding fish oil and increased threefold by oil supplied as bruised linseed compared with a megalac fat control. The greater effect of the linseed could be attributed either to the 30% higher intake of linoleic acid or the lower level of long-chain n-3 PUFA in the fish oil, 46g/day of EPA plus DHA, compared with 184 g α-linolenic acid added as the linseed supplement. The greater effectiveness of EPA plus DHA than α-linolenic acid on a weight basis may stem from their greater inhibition of biohydrogenation, a long observed phenomenon, or from direct availability as free oil compared with oil in linseed. The effectiveness of long-chain highly unsaturated fatty acids in raising CLA levels in milk was shown by supplementing cows diets with marine algae to give 2.95% fat with 6 - 7% total long-chain PUFA as 22:5 n-6 and 22:6 n-3 from Schizochytrium(Franklin *et al.*, 1999). CLA reached 2.62g/100g milk fat using unprotected algae compared with 0.37g/100g for the control diet.

Despite the extensive data indicating anti-carcinogenic activity, the role of CLA in preventing atherosclerosis is less clear. Lee *et al* (1994) using rabbits and Nicolosi *et al* (1997) using hamsters observed that CLA decreased plasma lipoproteins and development of atherosclerosis when the animals were fed cholesterol. In a mouse model the changes in blood lipids were also toward a less atherogenic profile but development of fatty streaks in the aorta increased (Munday *et al.*, 1999). Before we make great attempts to raise CLA levels in food these discrepancies must be resolved. Although the increased atherogenesis in the mice might be explained by alterations in the immune response and activation of macrophages, the action of CLA on components of the immune system is unclear (Hayek *et al.*, 1999) and differences between studies may depend in part on whether the concentration of arachidonic acid in tissues is decreased by CLA (Cook *et al.*, 1993).

A potential use for CLA in animal nutrition is to use it to partition energy toward protein deposition rather than lipogenesis. Dietary supplementation with 0.5% CLA decreased body fat of mice by over 50% within a 4 week period (Park *et al.*, 1997). Pigs fed 1.0% CLA over the last 40 days of finishing had a significant reduction in subcutaneous fat (-6.8%, $P<0.01$) and a small increase in lean (+2.3%, $P=0.02$) (Dugan *et al.*, 1997). However, although meat quality was not altered by feeding the CLA the level of intramuscular fat, assessed as marbling score or extractable lipid, increased from 390 to 434 and from 15.5 to 19.2g/kg respectively (Dugan *et al.*, 1999). Tissue contents of total CLA isomers expressed as % of total fatty acids were, for the triacylglycerol fraction, liver 6.0%, heart 3.6%, backfat 4.7% and omental fat 2.9%. Whereas the isomeric composition of the CLA in adipose tissue resembled that of the dietary CLA, the incorporation of different isomers into different phospholipids varied (Kramer *et al.*, 1998). Thus the overall efficiency of incorporation of CLA into tissue lipids is somewhat less than the incorporation of linoleic acid which at 1% of feed would reach concentrations of 10 - 15% in backfat. However, the levels of CLA in the tissues are higher than those obtained in modified milks and meats. Although taking a capsule of CLA may be more effective in supplying CLA to the human diet, using it to improve carcass quality and selling the meat suitably labelled would double the potential benefits.

Improving the fatty acid composition of pig lipids

The muscle and adipose tissue of pigs fed the normal European cereal based diets contain a relatively high level of linoleic acid at around 14% so that the P:S ratio is well above the minimum recommended (0.45) at approximately 0.6. However, the concentration of α-linolenic acid is low, giving an n-6:n-3 ratio of 10 in adipose tissue and 15 in muscle (Enser *et al.*, 1996). In muscle the ratio of arachidonic acid to EPA was also higher at 7, reflecting the differences in the concentrations of the precursor fatty acids. The addition of fish oil to pig feeds to increase the levels of EPA and DHA has been widely reported (Morgan *et al.*, 1992, Irie and Sakimoto, 1992; Ishida *et al.*, 1997; Leskanich *et al.*, 1997, Hürnberg *et al.*, 1999). Fish oil fed at approximately 1.0% of feed produced muscle lipid containing 1.0% of EPA and DHA and 0.4% - 0.5% of each of these fatty acids in adipose tissue (Leskanich *et al.*, 1997; Morgan *et al*, 1992). The increase in the percentage of EPA and DHA occurred mainly at the expense of arachidonic acid in both trials but other changes were small and inconsistent between trials. Although rapeseed oil was included in the trial by Leskanich *et al.*, and lowered the feed n-6: n-3 ratio from 8.96 to 4.89, the ratio in the *m.longissimus* only fell from 23 to 14 and in backfat from 11 to 7. In both trials, organoleptic assessment indicated that flavour was not affected by the increase in the levels of oxidatively unstable unsaturated fatty acids. A serving of meat based on 100g fresh muscle plus 10g adipose tissue would supply approximately 44% of the daily recommended intake of EPA plus DHA of 200mg. Although greater supplementation can produce higher tissue concentrations of these fatty acids, the organoleptic properties of the meat deteriorates (Overland *et al.*, 1996) with off odours and off flavours becoming significant with EPA at 1.5% and DHA at 1.8% of tissue fatty acids.

As a result of environmental and other concerns about the use of fish oil in animal feeds, many studies have been carried out to lower the n-6:n-3 ratio by feeding linseed (flaxseed) or canola as a source of α-linolenic acid (Cherian and Sim, 1995; Ahn, Lutz and Sim, 1996; Romans *et al.* 1995a, b; Spect-Overholt *et al.*, 1997); Riley *et al.* 1998a,b; Enser *et al.* 2000). The incorporation of dietary α-linolenic acid into porcine lipids is proportional to the amount in the feed and the time for which it is fed although the efficiency of deposition is approximately 25% less than for linoleic acid. Romans *et al* (1995b) observed that feeding 15% flaxseed, so that α-linolenic acid was 35% of dietary fatty acids increased the content in *m.longissimus* threefold when fed for 21 days before slaughter. EPA concentrations also increased and there were smaller increases in DHA. In backfat, α-linolenic acid reached 3% of total fatty acids. A similar amount of α-linolenic acid from flaxseed fed for the whole fattening period raised backfat α-linolenic to 14% and muscle levels to 8% of total fatty acids but increases in EPA and DHA in these tissues were small (Cherian and Sim, 1995). Not only did the long-term feeding fail to increase the concentrations of EPA and DHA but the concentrations of α-linolenic were well in excess of the 3% - 4% in adipose tissue which have been reported to cause flavour defects as a result of lipid oxidation (Romans *et al.* 1995b; Ahn *et al.*, 1996). However, when α-tocopheryl acetate was included in the feed at 100mg/kg α-linolenic acid at 3.9% of backfat lipid did not affect the flavour or odour of grilled steaks (Riley *et al.*, 1998a). Because the metabolism of linoleic acid and α-linolenic acid to longer-chain more unsaturated PUFA depends upon competition between the two for the same enzyme systems (Brenner, 1974), we attempted to increase tissue concentrations of longer-chain n-3 PUFA to a greater extent by decreasing the dietary concentration of linoleic acid from 15.5 to 10g/kg whilst increasing α-linolenic from 1.9 to 4g/kg. This gave 18:2 to 18:3 ratios in the diet of 8.2 and 2.5 respectively. The fatty acid composition of the meat from pigs fed the high amount of α-linolenic acid met all the desired criteria but one: concentrations of α-linolenic acid in adipose tissue were raised toward the target 3% level with significant increases in DPA and DHA the P:S ratio remained in the acceptable range and the n-6:n-3ratio fell toward desirable level of 4 (Enser *et al.*, 2000). The effects of diet on the fatty acids of the *m.longissimus* were similar to those in backfat: the 18:2 to 18:3 ratio was halved and EPA was significantly increased. Muscle neutral lipids showed few significant differences between treatments whereas in the phospholipids all n-3 PUFA were significantly raised by feeding linseed and all n-6 PUFA were decreased. Only the muscle18:2 to 18:3 ratio failed to reach the target of 4 although it fell by an average of 55. This clearly shows the possibility of changing pork fatty acids to meet the ideal composition

In this trial there were no significant effects of diet on meat quality assessed organoleptically or chemically in pork chops, liver, bacon and sausages after conditioning or frozen storage or under simulated retail display (Sheard *et al.* 2000). Clearly feeding α-linolenic acid can improve the value of pork in human nutrition by lowering the n-6:n-3 and 18:2 to 18:3 ratios. However, the endogenous synthesis and deposition of longer-chain n-3 PUFA is less than can be achieved by feeding those fatty acids preformed in fish oil. It remains to be determined whether a combination of fish oil and linseed feeding with lower dietary linoleic acid would produce meat of normal quality with more long-chain n-3 PUFA.

Conclusion

Lean ruminant meat from forage fed animals is a valuable source of long-chain n-3 PUFA in the human diet but can be improved by dietary manipulation without deleterious effects on flavour. However, it is difficult to modify the P:S ratio with most strategies currently available. In pigs dietary manipulation can result in meat with both a good P:S ratio and n-6: n-3 ratio. There is clearly potential to raise CLA levels in ruminants and pigs if it's value in human nutrition can be proved. Overall the results discussed indicate the potential to improve the overall quality of meat, a valuable and enjoyable component of the human diet.

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Table 1. Effects of dietary n-3 PUFA supplements on the fatty acid content (mg/100g) and flavour attributes of beef *m.longissimus*

	Dietary fat					
Fatty acid	Megalac	Linseed	Fish oil	Linseed./Fish oil	SED	P
18:1 trans	63	147	184	173	33.2	<0.01
18:2 n-6	81	78	66	64	9.2	NS
18:3 n-3	22	43	26	30	5.6	<0.01
20:4 n-6	23	21	14	17	1.5	<.001
20:5 n-3	11	16	23	15	1.9	<0.001
22:6 n-3	2.2	2.4	4.6	4.9	0.52	<0.001
Total fatty acids	3529	4222	4292	3973	741	NS
Flavour attributes* of grilled loin steaks						
Rancid	1.6^{ab}	0.4^{a}	2.2^{b}	1.1^{ab}	0.59	<.05
Fishy	5.6^{a}	4.9^{a}	9.7^{b}	4.9^{a}	1.37	<.001
Overall liking	29.0^{ab}	35.5^{c}	25.3^{a}	32.5^{bc}	2.18	<.001

*Unstructured line scales, 0-100, low attribute-high attribute