Effect of n-6:n-3 ratio of dietary oils and vitamin E on α-tocopherol content of liver, cardiac and skeletal muscle of pigs.

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Background

Supranutritional levels of dietary vitamin E have been shown to enhance the oxidative stability of pork meat, maintain colour stability and reduce drip loss (reviewed by Buckley and Morrissey, 1992; Wood and Enser, 1996). Replacement of beef fat, rich in saturated and monounsaturated fatty acids with soyabean oil rich in n-6 polyunsaturated fatty acids (PUFA) increased the C18:2/C18:1 ratio and the susceptibility of the pork meat to oxidation but this was reduced by supplementary vitamin E. Reduced incidence of coronary heart disease associated with consumption of fish has led to a recommendation to double the intake of long chain n-3 fatty acids (eicosapentaenoic acid EPA and docosahexaenoic acid DHA) in the diet of man (Department of Health, 1994) and prompted recent interest in improving the n-6:n-3 fatty acid ratio of meat as one means of accomplishing this. In addition, there is an inverse relationship between coronary heart disease and antioxidant intake, and vitamin E supplementation in particular (Diaz et al., 1997). The use of rapeseed or flaxseed as the source of 18:3 n-3 primarily increases 18:3 n-3 in adipose tissue with smaller increases in muscle, little change in EPA and DHA, has produced soft back fat and increased incidence of off flavours in taste panel tests (Shackelford et al., 1990; Gill et al., 1995; Romans et al., 1995ab). In contrast, the use of a small amount of fish oil incorporates substantial EPA and DHA into pig meat without any noticeable fat softening (Morgan et al, 1992; Leskanich et al., 1993, 1994). The inclusion of fish oil in the diet of rats, marmoset monkeys and pigs has indicated a reduced incidence of cardiac arrythmias (Charnock, 1991; McLennan et al., 1993; Hartog et al., 1987) and in a dog model infusion of fish oil fatty acids completely inhibited ischaemia-induced ventricular fibrillation (a cause of sudden death) during an exercise stress test (Billman et al., 1994). Consequently, dietary fish oil may be of direct benefit to the health of the pig. We have previously shown feeding chickens on fish oil or fish meal incorporates substantial EPA and DHA into the meat and that high levels of vitamin E will reduce oxidation of the muscle and maintain acceptable organoleptic scores (Huang & Miller, 1993a, 1994; Miller & Huang, 1993).

Objectives

The present study investigates the effect of different sources of n-3 fatty acid and of different ratios of n-6:n-3 fatty acids on fatty acid composition of pig tissues in the presence of normal and supranutritional amounts of vitamin E and the consequences for pig health determined in cardiac function tests. The results on tissue vitamin E and in vitro Fe-induced thiobarbituric reactive substances (TBARS) in cardiac muscle are given in this presentation.

Methods

Sixteen Large White x Landrace male pigs weighing 13.8±0.84kg were reared on diets containing 30 g/kg of one of 4 oils (beef dripping, maize oil, mackerel oil, rapeseed oil) with either a normal (50 mg/kg) or supranutritional (200 mg/kg) DL α-tocopheryl acetate to 40 kg live weight. A rearer diet with a high proportion of cooked cereals, whey and whey protein concentrate was used to 25 kg live weight followed by a grower diet with these ingredients replaced by uncooked cereals. After an over-night fast the animals were anaesthetised and cardiac function tests were carried out before the animals were killed by an overdose of anaesthetic. Cardiac tissue was sampled from an area of the left ventricle made ischaemic during the cardiac function tests and from a control non-ischaemic area. Tocopherols were extracted, along with pentamethyl-6-chromanol as internal standard for feeds and β-tocopherol for tissues, by the method of Ueda & Igarashi (1990) and redissolved in 200µl hexane for HPLC analysis on a Nucleosil $5NH_2$ column eluted with 7.5ml/l isopropanol in hexane and measured with a fluorescence detector at excitation 292nm and emission 330 nm. TBARS were measured by the method developed with chicken muscle by Huang & Miller, (1993b) except that Fe concentration had to be increased from 1.0mM to 4.0 mM before any TBARS could be produced with heart muscle.

Results and Discussion

The analysis of the diets (Table 1) indicated that the target difference of 137 mg added α-tocopherol (150 I.U. vitamin E) between the low and high levels of supplementation was generally achieved.

Table 1. Tocopherol content in experimental diets (mg/kg)

		Rearer					Grower				
		α–	β–	γ–	δ–	**total**	α–	β–	γ–	δ–	**total**
Beef fat	**50E**	43.0	0.7	7.7	1.3	**52.8**	66.3	0.9	8.6	0.6	**76.4**
	200E	185.9	0.6	7.6	1.2	**195.2**	222.8	1.0	9.4	0.8	**234.0**
Corn oil	**50E**	69.7	0.5	21.7	2.0	**93.8**	61.5	0.6	8.0	1.5	**71.6**
	200E	224.5	0.5	22.7	2.3	**249.9**	190.7	0.7	9.8	1.8	**202.9**
Fish oil	**50E**	54.5	0.7	7.3	0.7	**63.2**	63.5	0.6	5.7	0.6	**70.3**
	200E	199.4	0.5	5.2	1.2	**206.3**	165.0	0.7	6.1	ND	**171.8**
Rape oil	**50E**	54.1	ND	9.6	1.4	**65.2**	71.8	0.5	7.9	1.5	**81.7**
	200E	244.7	ND	9.7	1.3	**255.6**	212.0	0.5	7.9	1.6	**222.0**

ND Not detected

A paired t-test showed the ischaemic heart tissue had less α-tocopherol (Normal 9.06; Ischaemic 8.52 μg/ g tissue; P = 0.025, two tail test). There were no oil x E interactions so the main treatment effects are shown for each of the two heart tissues and also for L. dorsi muscle for comparison.

Table 2. α-tocopherol content of heart, skeletal muscle and liver (μg/ g tissue) and fasting plasma (μg/ ml).

	Beef	Corn	Fish	Rape	SED	50E	200E	SED
Normal heart	9.6	9.5	9.1	8.0	0.86	6.6j	11.6k	0.61
Ischaemic heart	9.3	8.8	8.2	7.9	0.89	6.4j	10.6k	0.63
L. dorsi muscle	2.63	2.54	2.27	2.35	0.239	1.59j	3.30k	0.169
Liver	12.6	14.3	8.2	7.3	3.43	5.1m	16.2n	2.42
Plasma	1.83a	1.53ab	1.25b	1.22b	0.193	0.93j	1.99k	0.137

Values with different letters (a, b) differ at P<0.05; (j, k) differ at P < 0.001; (m, n) differ at P <0.002.

Additional dietary vitamin E significantly increased the α-tocopherol content of fasting plasma, liver, heart and L. dorsi muscle. Heart muscle had 3.5 to 4.2 times more α-tocopherol than skeletal muscle. The dietary oils had no significant effect on the α-tocopherol content of the heart or skeletal muscle. The difference in heart tissue between rapeseed oil and beef dripping treatments approached significance at the P=0.1 level. A similar pattern was seen in the liver, with fish and rapeseed oils having non-significantly lower concentrations of α-tocopherol. In the liver, but not in cardiac or skeletal muscle, the difference was mainly in the high vitamin E groups where the concentrations were approximately 50 % of the corn oil and beef dripping values, but the diet x vitamin E interaction was not significant (P= 0.39). The fasted plasma concentration was significantly reduced for fish oil and rapeseed oil compared with beef fat with corn oil intermediate. The high content of α-tocopherol in cardiac muscle may reflect the greater need for antioxidant in this tissue. Heart tissue failed to show any oxidation in the original TBARS until the iron was increased from 1.0mM to 4.0mM. With this modification the fish oil-normal E treatment gave increased formation of TBARS but this was reduced to increments similar to that of the other oil treatments at the high vitamin E level (data not shown). Increased in vivo oxidation in tissues of PUFA from fish oil and rapeseed oil treatments may have caused loss of α-tocopherol but this would appear to have come mainly from liver reserves rather than from cardiac and skeletal muscle.

Conclusions

Different tissues maintain different levels of α-tocopherol which appear to be related to the oxidative activity of the tissue apart from stores in liver and adipose tissue. Plasma α-tocopherol reflected liver reserves but was not closely related to cardiac or muscle levels. Even a high commercial level of vitamin E supplementation (50 I.U./kg) does not maximise cardiac or skeletal muscle α-tocopherol. At 200 I.U./kg muscle levels appear to be saturated whereas liver stores increase. Increasing the dietary supply of n-3 PUFA has little effect on the α-tocopherol content of cardiac or skeletal muscle compared with either saturated/monounsaturated or n-6 PUFA but may reduce liver reserves.

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