Effect of Dietary Fat Quality and Alpha-Tocopheryl Acetate Supplementation on the Susceptibility of Porcine Tissue to Lipid Peroxidation

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SUMMARY

Dietary fat quality had a marked influence on the fatty acid composition of porcine muscle and liver. The ratio of C18:2/C18:1 was significantly higher in the muscle and liver from pigs fed the fresh soya oil diet compared to pigs fed the oxidized soya oil diet. Tissues from pigs fed the oxidized soya oil diet were also found to be more susceptable to lipid peroxidation than tissues from pigs fed fresh soya oil. Alpha-Tocopheryl acetate supplementation significantly reduced the susceptability to lipid peroxidation of liver and muscle from pigs fed either fresh or oxidized soya oil diets. It can be concluded that dietary fat quality may cause changes in the fatty acid composition leading to changes in the susceptability of tissue lipids to oxidative attack. However, dietary alpha-tocopherol acetate may offer an effective means of minimizing the adverse effects of dietary fat quality.

INTRODUCTION

Dietary fat quality influences the fatty acid composition of porcine muscle and adipose tissue (Marchello et al., 1983; Rhee et al. 1988). Changes in fatty acid composition may in turn be accompanied by changes in the susceptability of tissue lipids to oxidative attack (Rhee et al., 1988). Alpha-tocopheryl acetate supplementation of pig diets has been shown to result in increased alpha-tocopherol concentration in porcine tissues with a resultant increase in the stability of muscle (Monahan et al., 1989).

The objectives of this study were to investigate the effects of dietary fat quality and alpha-tocopheryl acetate supplementation on: (a) the fatty acid profiles of porcine muscle and liver (b) the susceptability of porcine muscle and liver to lipid oxidation and (c) the alpha-tocopherol levels in the various tissues.

MATERIALS and METHODS

Thirty-two Landrace X Large White pigs, weighing approximately 45 Kg liveweight were randomly divided into 4 groups of eight. Each group was fed a diet containing 3% fresh soya oil or 3% oxidized soya oil with either a basal level (10mg/Kg diet) or a supplemented level of alpha-tocopheryl acetate (200mg/Kg diet). The oxidized soya oil had a peroxide value of approximately 100meq O_2 /Kg oil. The pigs were given water and feed ad libitum and were slaughtered when they weighted in excess of 85Kg. At evisceration, liver samples were taken. The carcasses were then chilled overnight to 5°C and a sample of the Longissimus dorsi muscle was taken.

All samples were vacuum packed and stored at -20°C until required. Fatty acid methyl esters of the neutral and polar lipid fractions isolated from muscle and liver were prepared by the method of Slover and Lanza (1979) and Maxwell and Marmer (1983), respectively. The methyl esters of the fatty acids were analysed on a glass column, 2m X 3mm i.d. containing 10% Silar-10C on 100/120 mesh Gas-Chrom Q. The analysis was performed on a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector. A Shimadzu C-R6A Chromatopac intergrator was used for the calculation of peak areas. Alpha-tocopherol levels in liver and muscle were determined using the HPLC method of Buttriss and Diplock (1984). The stability of muscle tissue homogenates to iron-induced lipid peroxidation was derermined by a modification of the method of Kornbrust and Mavis (1980). 2-Thiobarbituric acid reactive substances (TBARS) was determined by the method of Buege and Aust (1978) and reported as nmoles malonaldehyde per mg of protein as outlined by Monahan et al. (1989).

RESULTS and DISCUSSION

Muscle tissue fatty acids: The neutral and polar lipid fractions isolated from the L. dorsi muscle of pigs receiving the 3% oxidized soya oil diet had a higher proportion of C18:1 and a lower proportion of C18:2 than the lipid fractions from pigs fed the 3% fresh oil diet. The differences were significant in all cases ($p < 0.05$) except for levels of C18:1 in muscle samples from pigs fed the oxidized soya oil diet with basal levels of alpha-tocopheryl acetate.
Both lipid fractions in liver from pigs fed the 3% oxidized soya oil diet had significantly higher levels of C18:1 ($p < 0.05$) and significantly lower levels of C18:2 ($p < 0.05$) than pigs fed the 3% fresh oil diet. In liver and muscle the ratio of C18:2/C18:1 was significantly lower ($p < 0.05$) in both the neutral and polar lipid fractions. Alpha-tocopheryl acetate supplementation reduced the effect of oxidized soya oil on fatty acid profiles. However, the effect was not significant. In the case of the neutral and polar fractions of liver and the polar fraction of muscle, the ration of unsaturated/saturated fatty acids was significantly higher ($p < 0.05$) in pigs fed the fresh oil diet.

Tissue alpha-tocopherol levels: The alpha-tocopherol levels were significantly lower ($p < 0.05$) in liver and muscle from pigs fed the oxidized soya oil diet compared to the fresh soya oil diet except for muscle from pigs fed the basal alpha-tocopherol diets (Table 1). These results are in agreement with those reported from experiments with chicks by Lin et al. (1989) and Sheehy et al. (1990). It is likely that hydroperoxide degradation products from the oxidized soya oil in the diet induced rapid oxidation of the membrane-bound lipids in the tissues. Thus, the lower alpha-tocopherol levels in tissues from pigs fed the the oxidized soya oil is due to its action as a free radical scavenger. Century and Horwitt (1959) observed a vitamin E deficiency disease, encephalomalacia, in broilers fed high levels of an oxidized diet.

Lipid oxidation in muscle and liver: Dietary alpha-tocopheryl acetate supplementation significantly increased ($p < 0.05$) the stability of liver and muscle tissues to lipid peroxidation after 160 min incubation, from pigs fed both the fresh and oxidized soya oil diets (Fig. 1). These results are in agreement with findings by Monahan et al. (1990).
Muscle and liver tissues from pigs fed the oxidized soya oil diets were more susceptable to lipid oxidation than tissues from pigs fed the fresh oil diets of comparable alpha-tocopheryl acetate supplementation except for muscle samples in pigs fed the high supplementation diet. The increased susceptability to lipid peroxidation in the oxidized soya oil groups may be due to the increased number of allyl groups present (Arkawa and Sagai, 1986). In addition the residual levels of alpha-tocopherol in liver and muscle tissues from pigs fed the oxidized soya oil diet may contribute to the increased susceptability to lipid peroxidation.

CONCLUSIONS

Feeding an oxidized soya oil diet to pigs had the effect of lowering the residual alpha-tocopherol levels in muscle and liver for both basal and supplemented diets. An increased susceptability to lipid oxidation was observed. Oxidized fat had a significant effect on the fatty acid profile resulting in decreased unsaturated to saturated and C18:2 to C18:1 ratios.

Table 1. Mean α-tocopherol content and standard error of the mean of liver and muscle of pigs fed fresh and oxidized soya oil diets with basal and supplemented levels of α-tocopheryl acetate.

Dietary treatment	α-tocopherol[1]	
	Liver (mg/g)	Muscle (mg/g)
Fresh soya oil:		
basal	11.52±0.76[a]	3.95±0.28[a]
supplement	98.57±6.15[b]	13.57±0.71[b]
Oxidized soya oil:		
basal	9.30±0.65[c]	3.33±0.41[a]
supplemented	82.03±3.19[d]	9.67±0.49[d]

[1] Mean values and standard error of the mean of six analyses
[a b c d] Means in the same column bearing different superscripts differ significantly ($p<0.05$)

Fig. 1: Effect of dietary fat quality and alpha-tocopheryl acetate supplementation on iron induced lipid peroxidation in pig muscle and tissue

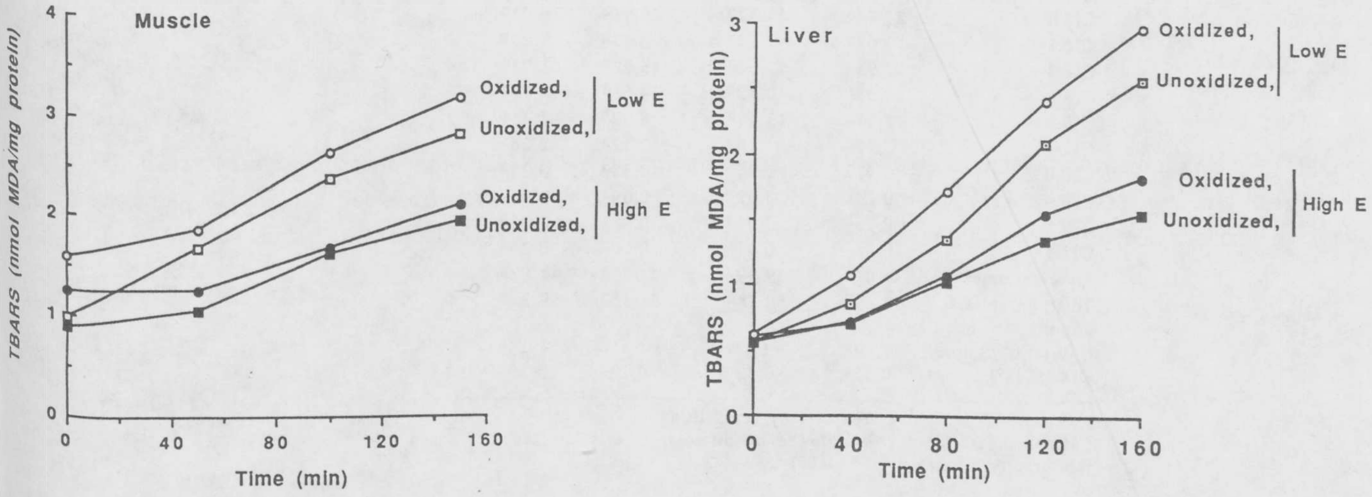

Table 2. Fatty acid profiles of the neutral lipid fraction of liver from pigs fed fresh soya oil and oxidized soya oil diets with control and supplemented levels of α-tocopheryl acetate.

	Fresh Soya oil[1,2]		Oxidized Soya oil	
Fatty acid	Control %	Supplemented %	Control %	Supplemented %
C14:0	0.52^{b}	0.57^{b}	1.53^{a}	1.39^{a}
C16:0	13.35^{a}	14.01^{a}	15.34^{a}	14.38^{a}
C16:1	1.83^{a}	1.74^{a}	0.92^{a}	1.14^{a}
C18:0	23.93^{a}	23.35^{a}	25.63^{a}	24.71^{a}
C18:1	12.99^{b}	13.53^{b}	15.79^{a}	14.71^{a}
C18:2	21.04^{a}	20.85^{a}	18.23^{b}	18.95^{b}
C18:3	1.27^{a}	1.07^{a}	0.73^{a}	1.01^{a}
C20:4	13.91^{a}	14.36^{a}	12.96^{a}	13.57^{a}
C20:5	1.79^{a}	1.62^{a}	0.94^{b}	1.09^{b}
C22:0	2.13^{a}	2.07^{a}	3.11^{a}	2.93^{a}
C22:5	3.82^{a}	3.91^{a}	2.52^{b}	3.11^{ab}
C22:6	2.94^{a}	2.88^{a}	1.63^{b}	2.23^{ab}
Total saturates	39.93^{b}	40.00^{b}	45.61^{a}	43.41^{a}
Total unsaturates	59.59^{a}	59.96^{a}	53.72^{b}	55.81^{b}
Ratio:				
Unsaturate/Saturate	1.49^{a}	1.50^{a}	1.18^{b}	1.29^{b}
C18:2/C18:1	1.62^{a}	1.54^{a}	1.15^{b}	1.29^{b}

[1] Percent of total peak area of fatty acids listed
[2] Means in the same row followed by different superscripts are significantly different ($p < 0.05$).

Table 3. Fatty acid profiles of the polar lipid fraction of liver from pigs fed fresh soya oil and oxidized soya oil diets with control and supplemented levels of α-tocopheryl acetate

	Fresh Soya oil[1,2]		Oxidized Soya oil	
Fatty acid	Control %	Supplemented %	Control %	Supplemented %
C14:0	0.71^{b}	1.04^{b}	1.51^{a}	1.41^{a}
C16:0	14.53^{a}	15.06^{a}	17.69^{a}	16.93^{a}
C16:1	0.93^{b}	1.57^{a}	0.53^{b}	0.92^{b}
C18:0	29.43^{a}	27.93^{a}	30.63^{a}	29.38^{a}
C18:1	12.23^{b}	11.68^{b}	13.99^{a}	14.06^{a}
C18:2	22.93^{a}	22.31^{a}	19.43^{b}	20.47^{b}
C18:3	1.32^{a}	0.93^{a}	0.62^{a}	0.89^{a}
C20:4	13.32^{a}	14.52^{a}	11.47^{b}	11.52^{b}
C20:5	0.93^{a}	0.63^{a}	0.43^{a}	0.56^{a}
C22:0	1.18^{c}	2.12^{bc}	2.59^{ab}	3.13^{a}
C22:6	2.10^{a}	1.34^{ab}	0.71^{b}	0.73^{b}
Total saturates	45.85^{b}	46.09^{b}	52.42^{a}	50.85^{a}
Total unsaturates	53.76^{a}	52.99^{a}	47.18^{b}	48.59^{b}
Ratio:				
Unsaturate/Saturate	1.17^{a}	1.15^{a}	0.90^{b}	0.96^{b}
C18:2/C18:1	1.87^{a}	1.91^{a}	1.39^{b}	1.46^{b}

[1] Percent of total peak area of fatty acids listed
[2] Means in the same row followed by different superscripts are significantly different ($p < 0.05$).

Table 4. Fatty acid profiles of the neutral lipid fraction of muscle from pigs fed fresh soya oil and oxidized soya oil diets with control and supplemented levels of α-tocopheryl acetate.

	Fresh Soya oil[1,2]		Oxidized Soya oil	
Fatty acid	Control %	Supplemented %	Control %	Supplemented %
C14:0	0.93^{a}	1.03^{a}	0.71^{a}	0.80^{a}
C16:0	23.74^{a}	24.32^{a}	26.41^{a}	26.01^{a}
C16:1	3.02^{a}	3.62^{a}	3.93^{a}	3.87^{a}
C18:0	13.93^{a}	13.69^{a}	13.92^{a}	13.93^{a}
C18:1	38.58^{b}	40.32^{b}	44.61^{a}	44.47^{a}
C18:2	13.57^{a}	13.47^{a}	8.02^{b}	8.43^{b}
C18:3	2.71^{a}	2.86^{a}	2.12^{a}	2.26^{a}
C20:0	0.41^{a}	0.35^{a}	0.39^{a}	0.28^{a}
C20:4	0.04^{a}	0.02^{a}	0.09^{a}	0.06^{a}
C20:5	0.03^{a}	0.04^{a}	0.08^{a}	0.05^{a}
C22:6	0.38^{a}	0.41^{a}	0.16^{a}	0.23^{a}
Total saturates	39.01^{a}	39.39^{a}	41.43^{a}	41.02^{a}
Total unsaturates	58.33^{a}	60.74^{a}	58.56^{a}	59.37^{a}
Ratio:				
Unsaturate/Saturate	1.49^{a}	1.54^{a}	1.41^{a}	1.45^{a}
C18:2/C18:1	0.35^{a}	0.33^{a}	0.18^{b}	0.19^{b}

[1] Percent of total peak area of fatty acids listed
[2] Means in the same row followed by different superscripts are significantly different ($p < 0.05$).

Table 5. Fatty acid profiles of the polar lipid fraction of muscle from pigs fed fresh soya oil and oxidized soya oil diets with control and supplemented levels of α-tocopheryl acetate

Fatty acid	Fresh Soya oil[1,2] Control	Fresh Soya oil[1,2] Supplemented	Oxidized Soya oil Control	Oxidized Soya oil Supplemented
	%	%	%	%
C14:0	1.15[b]	0.91[b]	1.94[a]	1.31[ab]
C16:0	17.51[b]	18.32[ab]	20.48[a]	19.92[a]
C16:1	1.46[a]	1.05[a]	1.53[a]	1.78[a]
C18:0	14.13[b]	15.59[ab]	17.43[a]	18.10[a]
C18:1	15.68[b]	14.38[b]	18.62[a]	19.32[a]
C18:2	36.23[a]	35.47[a]	30.48[b]	29.48[b]
C18:3	3.23[a]	2.97[ab]	2.37[ab]	2.02[b]
C20:4	7.58[a]	7.99[a]	5.26[b]	6.47[ab]
C20:5	1.23[a]	1.43[a]	0.53[b]	0.62[b]
C22:0	0.01[a]	tr[a]	0.03[a]	0.04[a]
C22:6	1.08[a]	1.22[a]	0.59[a]	0.44[a]
Total saturates	32.80[b]	34.82[b]	39.88[a]	39.37[a]
Total unsaturates	66.49[a]	64.51[a]	59.35[b]	60.13[b]
Ratio:				
Unsaturate/Saturate	2.03[a]	1.85[a]	1.49[b]	1.53[b]
C18:2/C18:1	2.31[a]	2.47[a]	1.64[b]	1.53[b]

[1] Percent of total peak area of fatty acids listed

[2] Means in the same row followed by different superscripts are significantly different ($p < 0.05$).

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