THE EFFECT OF DIETARY α-TOCOPHEROL SUPPLEMENTATION ON α-TOCOPHEROL LEVELS IN THE PIG AND ON OXIDATION IN PORK PRODUCTS.

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INTRODUCTION

Lipid oxidation is one of the primary factors limiting the shelf-life of cooked meats during storage (Gray and Pearson, 1987). Products of the auto-oxidation of unsaturated fatty aids adversely affect flavour, colour, nutritive value (Pearson et al., 1983) and also the safety of meats (Addis, 1986).

It is now well recognised that the polyunsaturated fatty acids, which are essential components of subcellular membranes, are primarily responsible for the initial rapid development of oxidized flavours in raw and cooked meat products (Younathan, 1985; Gray and Pearson, 1987). Rupturing of the compartmentalized cellular system of muscle, particularly by mincing or restructuring, results in the exposure of the labile phospholipid components to a pro-oxidative environment and facilitates the formation of free radicals capable of propagating the oxidative reaction.

Lipid oxidation is catalysed by myoglobin, haemoglobin and cytochromes (Tappel, 1962). However, free iron, released from myoglobin during cooking, appears to be the key pro-oxidant in cooked meats (Pearson et al., 1983). While there is considerable interest in the mechanism of lipid peroxidation, major attention is now focused on the methods of preventing off-flavour development and extension of shelf-life. The role of various exogenous anti-oxidants has been examined and reviewed (Gray and Pearson, 1987) and in recent times dietary α-tocopherol has been shown to improve the oxidative stability of muscle systems (Buckley and Connolly, 1980).

The objectives of the present study were to investigate the effect of long-term dietary α-tocopherol supplementation on (i) the α-tocopherol status of the pig; (ii) the susceptibility of muscle and other tissues to oxidation; and (iii) the oxidative stability of raw and cooked pork products during storage.

MATERIALS AND METHODS

Thirty-two Landrace x Large White pigs, weighing approximately 7 kg each at weaning, were randomly divided into two groups of sixteen (8 male, 8 female). One group was fed a control diet (30 mg α-tocopherol acetate/kg feed) and the other group was fed a diet supplemented with α-tocopherol (200 mg/kg feed) in the form of α-tocopherol acetate from the time of weaning to slaughter. The pigs were given water and feed ad libitum. The average weight of the pigs at slaughter was 84 kg. Blood and tissue (L. Dorsi muscle, liver, lung, heart, kidney) samples were taken at slaughter. The plasma fraction and tissues were stored at -20°C until required. α-Tocopherol in plasma and tissue samples was determined by the HPLC methods of Bieri et al. (1979) and Buttriss and Diplock (1984), respectively. The extent of lipid oxidation in meat products was assessed by the 2-thiobarbituric acid method of Ke et al. (1977) Thiobarbituric acid-reacting substances (TBARS) numbers were expressed as mg malonaldehyde per kg of tissue. Mitochondrial and microsomal subcellular fractions were isolated from muscle by differential centrifugation (Asghar et. al., 1988). The lability of the liver, lung, heart and kidney tissue homogenates, and the subcellular fractions of muscle, to iron-induced lipid peroxidation was determined by a modification of the method of Kornbrust and Mavis (1980). TBARS were determined by the method of Buege and Aust (1973) and reported as nmoles malonaldehyde per mg of protein.

RESULTS AND DISCUSSION

α-Tocopherol Levels

The average α-tocopherol levels of plasma and tissues of pigs fed the control diet and diet supplemented with α-tocopherol are presented in Table 1. The mean α-tocopherol level in plasma was significantly ($p < 0.01$) influenced by diet and increased from 2.03 to 5.48 $\mu g\ ml^{-1}$ on increasing the dietary level to 200 mg/kg feed. The mean α-tocopherol levels of muscle, liver, lung, heart and kidney increased 2.9, 2.5, 2.5, 2.6 and 2.7 - fold, respectively, in pigs receiving the high dietary α-tocopherol level.

Muscle TBARS numbers.

The TBARS results indicate that pork patties, both raw and cooked, from pigs fed the α-tocopherol - enriched diet were more stable than those from the control pigs (Tables 2 and 3). Raw pork patties from pigs fed high levels of α-tocopherol had significantly lower ($p < 0.01$) TBARS numbers than those from the control pigs after 9 days of storage. The TBARS data (Table 3) also showed that cooked pork patties from the α-tocopherol - supplemented group had consistently lower TBARS numbers than cooked patties from the

control group of pigs. Significant differences ($p<0.01$) in TBARS were observed after storage for 48h at 4°C. Oxidation during storage occurred much more readily in all the cooked samples in contrast to the more slowly developing rancidity in the raw patties. Similar relationships have been observed in broiler meat (Lin et al., 1988).

Iron-induced lipid peroxidation

Figure 1 shows the rates of iron-induced lipid peroxidation in liver, lung, heart and kidney tissue of pigs fed the control diet and the α-tocopherol - supplemented diet. Significant reductions ($p<0.01$) in the rates of iron-induced lipid peroxidation were observed in liver, heart and kidney tissue from the high α-tocopherol group (200 mg/kg feed) when compared to the control group. The higher levels of α-tocopherol in the tissue of pigs fed the α-tocopherol - enriched diet (Table 1) appeared to protect these tissues agaist peroxidation. Lung tissue from both the control and experimental group was resistant to iron-induced lipid peroxidation and no significant difference between groups was observed. The resistance of lung tissue to lipid peroxidation has been reported previously in the rat (Kornbrust and Mavis, 1980) and in the chicken (unpublished results from our laboratory) and is the subject of further investigation in our laboratory.

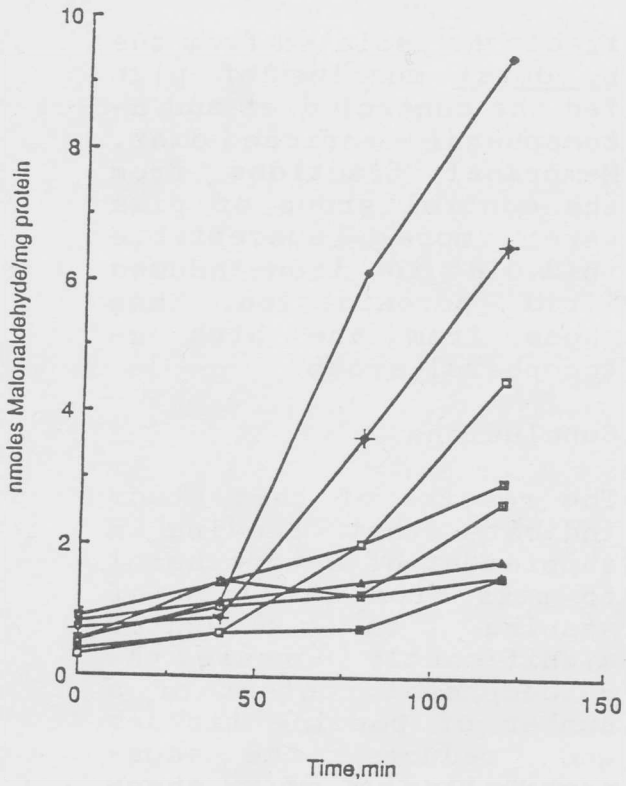

Figure 1. Effect of dietary α-tocopherol supplementation on the susceptibility of porcine tissues to iron-induced lipid peroxidation. ♦,liver (30 mg α-tocopherol/kg feed); ■,liver (200 mg α-tocopherol/kg feed); +,heart (30 mg α-tocopherol/kg feed); ▪,heart (200 mg α-tocopherol/kg feed); □,kidney (30 mg α-tocopherol/kg feed); ▪,kidney (200 mg α-tocopherol/kg feed); ▲,lung (30 mg α-tocopherol/kg feed); ▲,lung (200 mg α-tocopherol/kg feed).

Membranal lipid peroxidation

α-Tocopherol was found to be ~6 times more concentrated in muscle mitochondrial and microsomal fractions than in the tissue homogenate (Table 4). The concentration of α-tocopherol in the muscle membranal fractions of pigs fed the α-tocopherol - enriched diet was ~2.6 - fold higher than that of pigs fed the control diet. Figure 2 shows the rate of iron-induced lipid peroxidation in the mitochondrial and microsomal

fractions isolated from the L. dorsi muscles of pigs fed the control diet and α-tocopherol - enriched diet. Membranal fractions from the control group of pigs were more susceptible ($p<0.01$) to iron-induced lipid peroxidation than those from the high α-tocopherol group.

Conclusions

The results of this study indicate that feeding a supplement of α-tocopherol to pigs from the time of weaning to slaughter significantly increases the α-tocopherol content of a number of porcine tissues and reduces the susceptibility of these tissues to oxidative deterioration. In the case of muscle tissue, incorporation of α-tocopherol into the membranes stabilizes the highly unsaturated membranal phospholipids and significantly increases the oxidative stability of raw and cooked products.

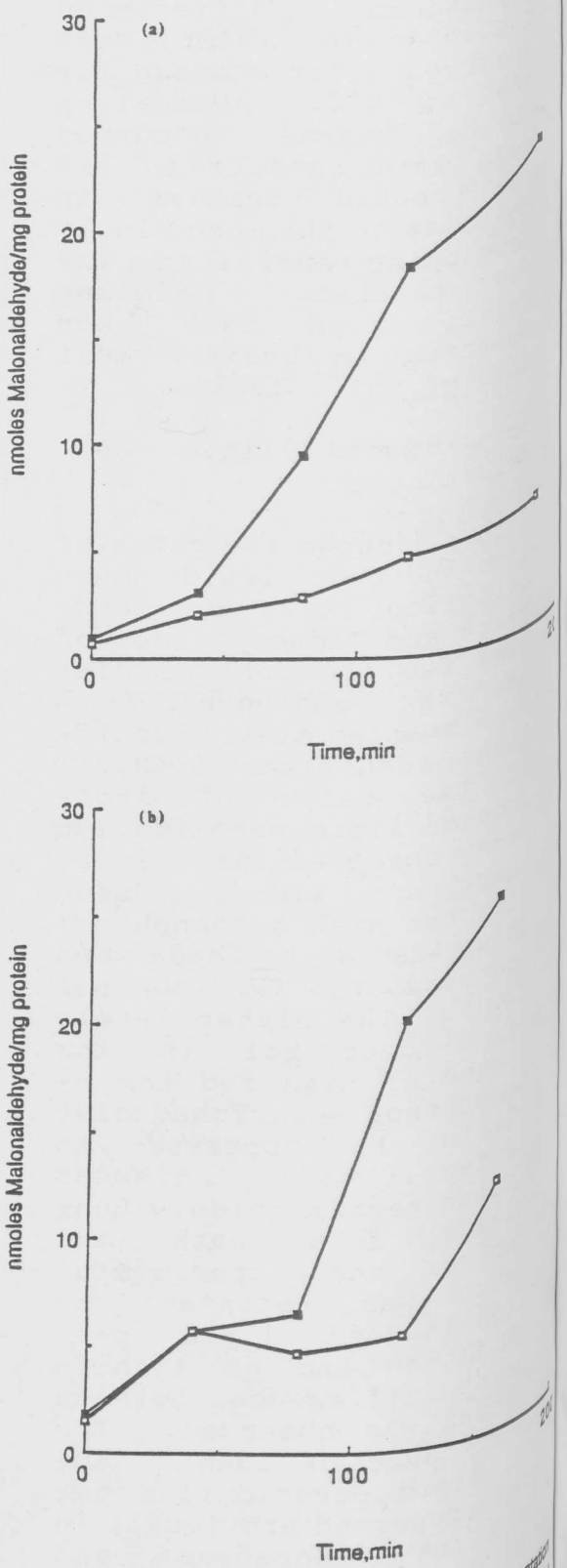

Figure 2. Effect of dietary α-tocopherol supplementation on iron-induced lipid peroxidation in the mitochondrial (a) and microsomal (b) fractions of muscle tissue. ■,(30 mg α-tocopherol/kg feed); ○,(200 mg α-tocopherol/kg feed).

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Table 1: Mean α-tocopherol content and standard error of the mean of plasma and tissue samples of pigs maintained on low (30 mg/kg feed) and high (200 mg/kg feed) levels of α-tocopherol acetate.

Item	Dietary α-tocopherol acetate	
	30 mg/kg feed	200 mg/kg feed
Plasma α-tocopherol (mg ml^{-1} plasma)	2.0 ± 0.2[a]	5.5 ± 0.4
Tissue α-tocopherol (ng mg^{-1} protein)		
Muscle	6.9 ± 0.4[a]	20.6 ± 2.3
Lung	22.5 ± 3.3[b]	55.3 ± 15.0
Liver	34.0 ± 6.8[b]	85.8 ± 11.1
Heart	25.3 ± 1.2[a]	65.3 ± 10.4
Kidney	9.0 ± 0.9[a]	24.7 ± 2.4

[a] α-Tocopherol levels differ significantly ($p<0.01$) between treatments

[b] α-Tocopherol levels differ significantly ($p<0.05$) between treatments

Table 2: Effect of dietary α-tocopherol supplementation on the TBARS numbers of raw pork patties during refrigerated storage at 4°C for up to 9 days.

Treatment	Storage time, days			
	0	3	6	9
Control diet	0.18	0.19	0.21	0.89
Supplemented diet (200 mg/kg feed)	0.13	0.21	0.20	0.45

Table 3: Effect of dietary α-tocopherol supplementation on the TBARS numbers of pork patties measured immediately after cooking, and after holding at 4°C for up to 6 days.

Treatment	Storage time, days			
	0	2	4	6
Control diet	0.27	5.00	5.22	6.83
Supplemented diet (200 mg/kg feed)	0.24	2.89	4.01	4.57

Table 4: The effect of dietary α-Tocopherol supplementation on the α-tocopherol content of the subcellular fractions of muscle tissue.

Fraction	α-Tocopherol, ng/mg protein [a]	
	control diet (30 mg/kg)	supplemented diet (200 mg/kg)
Homogenate	6.9 ± 0.4	20.6 ± 2.3
Mitochondria	40.9 ± 9.4	112.3 ± 18.4
Microsomes	56.4 ± 3.4	148.0 ± 7.9

[a] Mean values and SEM of six analyses