Influence of vegetable oils and alpha-tocopheryl acetate supplementation in lipid peroxidation in chick muscle

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SUMMARY

Chicks were fed fresh, heated or alpha-tocopheryl acetate supplemented heated vegetable oils and their effects on the alpha-tocopherol status, fatty acid composition and oxidative stability of thigh and breast muscle were determined. Dietary alpha-tocopheryl acetate concentration significantly influenced alpha-tocopherol concentrations in tissues. Plasma alpha-tocopherol was directly correlated with alphal-tocopherol concentrations in thigh and breast muscle. The fatty acid profiles of muscle lipids were altered considerably by modification of dietary lipids. The consumption of heated sunflower and linseed oils reduced alpha-tocopherol status, altered fatty acid composition of muscle lipids and increased susceptibility to lipid oxidation. Supplementation of diets containing heated oils with alpha-tocopheryl acetate resulted in some improvement of these parameters. The results suggest that oxidation products in thermally oxidized oilds may be absorbed, and catalyze lipid peroxidation *in vivo*.

INTRODUCTION

Lipid oxidation is a major cause of quality deterioration in poultry products, resulting in the production of undesirable odours and flavours and shortened shelf-life. A number of factors promote lipid oxidation in meat, including alpha-tocopherol and selenium deficiencies (Marusich et al. 1975; Combs & Regenstein, 1980), high concentrations of polyunsaturated fatty acids (PUFAs) in muscle lipids (Sklan et al., 1983; Lind et al. 1989) and cooking (Pikul et al. 1984).
A factor which has received less attention is the presence of oxidized fats in the diet. Severe feed oxidation induces nutritional encephalopathy (NE) in chicks. Synthetic antioxidants such as butylated hydroxytoluene (BHT) are usually added to feeds to prevent oxidative deterioration. However, their biological activity is uncertain. Feeding oxidized safflower oil or its methyl esters induced NE in chicks, despite the presence of BHT in the diet (Budowski et al. 1979). Synthetic oxidation products of oleic and linoleic acids, and a crude polar lipid extract of oxodized safflower oil methyl esters significantly increased the incidence of the disease. Chicks fed heated sunflower oil diets, containing BHT, and supplemented with alpha-tocopheryl acetate to control levels, had significantly lower alpha-tocopherol concentrations in several tissues including breast and thigh muscle than chicks fed fresh oil (Sheehy et al. 1990). These findings suggest that certain oxidation products may be absorbed from thermally oxidized oils and may catalyze peroxidation reaction *in vivo*.
The objective of this study, therefore, was to investigate the effects of fresh, heated or alpha-tocopheryl acetate supplemented heated vegetable oils on the growth and alpha-tocopherol status of chicks, and on the fatty acid composition and oxidative stability of muscle lipids.

MATERIALS and METHODS

Animals diets

Forty eight one-day old female ISA Brown chicks were purchased from a commercial hatchery. Chicks were randomly assigned to 6 groups and fed diets containing 8% fresh sunflower oil (FSO), fresh linseed oil (FLO), heated sunflower oil (HSO), heated linseed oil (HLO), alpha-tocopheryl acetate supplemented heated sunflower oil (HSE) or alpha-tocopheryl acetate supplemented heated linseed oil (HLE). Diets were prepared weekly, stored at 4°C and contained 0.005% BHT. Diets were adjusted to contain 50 mg alpha-tocopherol/kg, except for HSO and HLO which did not contain alpha-tocopherol. After 50 days feeding, chicks were killed by cervical dislocation. Blood was collected and plasma prepared by centrifugation. Thigh and breast muscle were dissected and quickly frozen in liquid nitrogen. Plasma and tissues were stored at -20°C until required.

Preparation of heated oils

Sunflower and linseed oils were heated in stainless steel containers in an oil bath at 140°C for 24 h. Oils were constantly aerated. Peroxide values of sunflower and linseed oils increased to maximum values of ca. 55 meq/kg during the first 5-10 h

of heating, and gradually declined, presumably due to the breakdown of hydroperoxides to secondary oxidation products. Fatty acid analysis of fresh and heated oils by capillary G.C. revealed that the linoleic acid (18:2) content of sunflower oil was reduced from 59.2% to 44.5% by heating for 24h, while the concentration of linolenic acid (18:3) in linseed oil decreased from 59.5% to 44.5%. Heated oils contained no alpha-tocopherol.

Analytical methods

Peroxide values of fresh and heated oils were determined by Lea's Rapid methods. The fatty acid composition of diet and muscle lipids was determined by the methods of Burton et al. (1985) and Slover & Lanza (1979). Plasma and muscle alpha-tocopherol concentrations were determined as previously described (Sheehy et al. 1991) except that a fluorescence detector was used for muscle alpha-tocopherol analyses. The concentration of thiobarbituric acid-reacting substances (TBARS) in plasma was determined by a spectrofluorometric method (Yagi 1984). The susceptibility of thigh and breast muscle to lipid oxidation during incubation with iron-ascorbate was measured by a modification of the method of Kornbrust & Mavis (1980). After incubation, TBARS were quantified by the method of Beuge & Aust (1978).

Statistical analysis

The statistical significance of the difference between means was determined by the t-test using the Minitab Statistical Package (Ryan et al. 1986).

RESULTS and DISCUSSION

Consumption of HSO and HSE diets by chicks resulted in a slight but not significant depression in body weight, relative to chicks fed FSO. However, growth was significantly depressed by feeding HLO and HLE, compared to the group fed FLO. Plasma alpha-tocopherol was directly correlated with alpha-tocopherol concentrations in thigh ($r=0.79$, $p<0.05$) and breast ($r=0.92$, $p<0.05$) muscle (Table 1). As expected, chicks consuming FSO or FLO had significantly greater ($p<0.05$) alpha-tocopherol concentrations in tissues than those fed HSO or HLO, due to the destruction of alpha-tocopherol in the oils by heating. Tocopherol concentrations in tissues of HSE and HLE-fed chicks were significantly higher ($p<0.05$) than those of chicks fed unsupplemented diets. However alpha-tocopherol concentrations were significantly lower than those of chicks fed fresh oils. This finding is important in view of the role played by the vitamin in the protection of meat against oxidative deterioration during storage, and the unsaturated nature of oils fed to poultry. These, and previous (Sheehy et al. 1990) results support the proposal that some oxidation products in heated oils may be absorbed and consume alpha-tocopherol *in vivo*.

Plasma TBARS were significantly elevated ($p<0.05$) in chicks fed HSO and HSE, compared to FSO-fed chicks (Table 2). Although a similar trend was evident in chicks fed the corresponding linseed oil diets, the differences were not significant. Basal TBARS concentrations in thigh and breast muscle of chicks fed HSO were significantly higher (0.05) than those in chicks fed HSE and FSO. Following stimulation of peroxidation with iron ascorbate, muscle from the HSO group was most susceptible to peroxidation, followed by that of the HSE group. Muscle from chicks fed FSO was most stable.

Differences in the oxidative stability of muscle from chicks fed linseed oil-based diets were less pronounced, although similar trends were evident. In general, the results indicate that high dietary alpha-tocopheryl acetate concentrations are associated with lower TBARS numbers.

Muscle from chicks fed linseed oil was less stable to iron-ascorbate induced peroxidation than that from sunflower oil-fed chicks. The fatty acid profiles of total lipids of thigh and breast muscle show that total PUFAs were significantly greater in chicks fed linseed oil-based diets, reflecting dietary lipids (Table 3). Chicks fed sunflower oil accumulated 18:2, while those fed linseed oil had high concentrations of 18:3 in muscle. However, the fatty acid profiles were significantly influenced by heating. Chicks fed FSO had significantly lower ($p<0.05$) quantities of 16:0, 16:1 and 18:1 and higher 18:2 and total PUFASs in thigh muscle than those fed HSO or HSE, while those fed FLO showed a significant reduction in 18:1 and increasess in 18:3 and total PUFAs, compared to chicks consuming HLO of HLE. Similar trends were apparent in breast muscle, although the changes were less pronounced. However, significant increases in 18:2 or 18:3 were observed after consumption of fresh rather than heated sunflower of linseed oils. This effect may be due, at least in part, to losses of these fatty acids during heating.

Table 1 α-tocopherol concentrations in plasma and muscle of chicks fed heated or unheated vegetable oils.

Tissue	α-tocopherol concentration[1]						r
	Sunflower oil			Linseed oil			
	HSO	HSE	FSO	HLO	HLE	FLO	
Plasma	0.80^{c}	6.58^{b}	19.1^{a}	0.65^{c}	7.84^{b}	11.3^{a}	-
Thigh muscle	1.64^{c}	7.71^{b}	17.8^{a}	1.14^{c}	10.9^{b}	19.8^{a}	0.79
Breast muscle	1.75^{c}	5.81^{b}	12.5^{a}	2.50^{c}	7.03^{b}	9.35^{a}	0.92

[1] µg/ml plasma or µg/g wet weight

Values are means of observation from 5 chicks.
a-c for each oil. mean values in a horizontal row not sharing a common superscript letter are significantly different ($p<0.05$).

Table 2 Effect of feeding heated or unheated vegetable oils on the concentrations of TBA-reactive substances (TBARS) in chicken tissues.

Tissue	Incubation time[2] (min)	TBARS[1]					
		Sunflower oil			Linseed oil		
		HSO	HSE	FSO	HLO	HLE	FLO
Plasma	0	8.33^{a}	7.91^{a}	2.70^{b}	9.66^{a}	8.00^{a}	4.85^{a}
Thigh	0	1.36^{a}	0.88^{b}	0.67^{b}	1.65^{a}	2.07^{a}	2.12^{a}
Muscle	40	6.29^{a}	1.31^{b}	0.57^{b}	8.83^{a}	5.15^{b}	5.74$^{a b}$
	80	9.25^{a}	3.56^{b}	1.77^{b}	12.9^{a}	6.66^{b}	8.04$^{a b}$
	120	10.7^{a}	6.54^{b}	3.44^{b}	14.0^{a}	11.4^{a}	10.6^{a}
Breast	0	1.08^{a}	0.47^{b}	0.51^{b}	0.69^{a}	0.37^{b}	0.42$^{a b}$
muscle	40	1.41^{a}	0.80^{b}	0.76^{b}	2.76^{b}	1.48^{a}	1.49^{a}
	80	2.08^{a}	0.82^{a}	0.62^{a}	3.27^{a}	1.69^{b}	1.58^{b}
	120	2.25^{a}	0.80^{a}	0.50^{b}	4.12^{a}	1.31^{b}	1.18^{b}

a-c For sunflower or linseed oil. values in horizontal rows not sharing a common superscript letter are significantly different ($p<0.05$).

Values are means of observations from 5 chicks

1 nmol MDA/ml plasma or nmol MDA/mg protein.

2 Time @ 37°C in the presence of iron/ascorbate.

TABLE 3: Effect of feeding various heated and unheated oils to chicks on the fatty acid Composition of muscle lipids.

Tissue	Diet	Fatty Acid (%)									
		16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5	22:5	22:6	Total PUFA
Thigh muscle	HSO	18.4^{a}	3.73^{a}	9.15	34.1^{a}	28.1^{b}	0.76	3.68	0	0.06	0.32	33.9^{b}
	HSE	18.8^{a}	3.08^{a}	10.2	32.0^{a}	30.0^{b}	0.59	3.36	0	0.05	0.19	35.1^{b}
	FSO	15.1^{b}	1.97^{b}	8.72	27.9^{b}	40.4^{a}	0.73	2.86	0	0.19	0.34	45.6^{a}
	HLO	15.6	1.56^{b}	10.2	29.5^{a}	18.3	18.8^{b}	1.62	0	1.41	0.95	42.1^{b}
	HLE	17.9	2.47^{a}	8.61	30.5^{a}	15.5	20.3^{b}	1.09	0	1.24	0.63	39.6^{b}
	FLO	13.1	1.46^{B}	7.48	25.7^{b}	17.5	30.7^{a}	1.02	0	0.99	0.65	51.7^{a}
Breast muscle	HSO	23.3	0.86	16.8	37.4^{ab}	11.3^{b}	0.26	3.59	0	0.32	0.69	17.8^{b}
	HSE	18.0	0.65	14.5	42.6^{a}	14.0^{ab}	0.09	2.91	0	0.09	0.82	19.6^{b}
	FSO	15.6	0.45	13.2	36.8^{b}	18.8^{a}	0.25	4.34	0	0.41	0.70	26.4^{a}
	HLO	22.0^{ab}	0.56	13.8	32.8	10.4^{ab}	6.21^{b}	1.83	1.63	3.20	1.56	25.0^{ab}
	HLE	24.3^{a}	0.53	12.3	33.8	10.1^{b}	4.53^{b}	1.62	1.49	3.26	1.28	22.3^{b}
	FLO	17.2^{b}	0.72	11.8	32.2	11.9^{a}	12.1^{a}	1.91	1.82	3.76	1.42	33.2^{a}

a - c For each tissue. and each oil. values within a column not sharing a common superscript letter are significantly different ($P<0.05$)
Values are means of observations from 5 chicks
Small amounts of other fatty acids have not been included in the table.

CONCLUSION

The consumption of heated sunflower and linseed oils by chicks caused some growth depression, reduced alpha-tocopherol status, altered the fatty acid composition of thigh and breast muscle lipids and was associated with increased susceptibility to lipid oxidation. Supplementation of diets containing heated oils with alpha-tocopheryl acetate resulted in partial stabilization of muscle lipids against oxidation, but fatty acid profiles were not altered significantly, and alpha-tocopherol status was reduced compared to controls fed fresh oils. The results indicate that caution should be exercised in the use of oxidized oils in the feeding of poultry if undesirable changes in carcass lipids are to be avoided. The use of synthetic antioxidants to stabilize oxidizing oils may not prevent such changes.

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