Consumption of thermally-oxidized sunflower oil by chicks reduces α-tocopherol status and increases susceptibility of tissues to lipid oxidation

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The effect of heated sunflower oil consumption on α-tocopherol status, fatty acid composition and oxidative stability of chicken tissues was investigated. Chicks were fed on diets containing (g/kg): fresh sunflower oil (FSO) 40, heated sunflower oil (HSO) 40 or heated sunflower oil (40) supplemented with α-tocopheryl acetate (HSE) to a similar α-tocopherol concentration as the FSO diet. Concentrations of α-tocopherol in tissues of chicks fed on HSO and HSE were significantly lower than those of chicks fed on FSO. Significant correlations were observed between plasma α-tocopherol concentration and the α-tocopherol concentrations of other tissues ($r \geq 0{\cdot}67$, $P < 0{\cdot}005$) and between log plasma α-tocopherol and plasma thiobarbituric acid-reacting substances (TBARS) concentrations ($r -0{\cdot}851$, $P < 0{\cdot}001$). The concentrations of TBARS in tissues of chicks fed on the various diets were generally very similar before stimulation of peroxidation with Fe–ascorbate. Susceptibility of tissues to Fe–ascorbate-induced lipid peroxidation was increased by feeding HSO. Supplementation with α-tocopheryl acetate reduced susceptibility to lipid oxidation to varying degrees, depending on the tissue. The results suggest that chronic ingestion of oxidized lipids may compromise free-radical-scavenging activity *in vivo* by depleting α-tocopherol in the gastrointestinal tract, or possibly in plasma and other tissues.

Heated oils: α-Tocopherol: Lipid peroxidation: Chick

The safety of thermally-abused fats and oils for human and animal consumption has been the subject of research over many years. Although feeding oxidized lipids to different species can result in a wide variety of symptoms including reduced appetite, growth depression, diarrhoea, histological changes in tissues and even death (Izaki *et al.* 1984), the general consensus has been that consumption of thermally-abused fats at realistic levels is not harmful since the dietary intake of lipid oxidation products is likely to be low (Artman, 1969). In recent years this suggestion has been criticized on the basis that in many earlier studies the pathological end-points assessed were crude, and fatty acid oxidation products and cholesterol oxides have been implicated in mutagenesis and carcinogenesis, and in the aetiology of coronary heart disease (Addis & Warner, 1991). There is considerable uncertainty over the degree to which lipid hydroperoxides and other oxidation products such as aldehydes, ketones, polymers and epoxides are absorbed from the intestine. Glavind *et al.* (1971) failed to observe peroxides in the lymphatic system of rats fed on oxidized lipids. However, Kanazawa *et al.* (1985) showed that some secondary oxidation products (but not hydroperoxides) are absorbed by rats and are taken up by the liver. Increased urinary excretion of malondialdehyde by rats following an oral dose of the enolic sodium salt was taken as indirect evidence of malondialdehyde absorption (Draper *et al.* 1984). Naruszewicz *et al.* (1987) observed a marked increase in plasma thiobarbituric acid-reacting substances (TBARS) in human subjects after the ingestion of thermally-oxidized

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soya-bean oil, while elevations in plasma TBARS after ingestion of fresh oil were minimal. These findings suggest that many lipid oxidation products may be absorbed by humans and animals. The fate of lipid oxidation products once formed in plasma or once they have been taken up by plasma is mainly unexplored (Frei *et al.* 1988). For these reasons, there is a need for further research into the biological effects of consumption of foods containing high levels of lipid oxidation products.

Fat blends for animal feeding may be based on waste fats from frying operations, or on byproducts such as distillation residues from edible-oil refining (Wiseman, 1986). In the broiler feed industry oxidation of feed ingredients can be costly to growers (Calabotta & Shermer, 1985). A low level of feed oxidation causes growth depression in broilers (Calabotta & Shermer, 1985; Lin *et al.* 1989), while feeding severely-oxidized vegetable oils containing high levels of linoleic acid to broilers may cause nutritional encephalopathy (NE) (Budowski *et al.* 1979, 1980). α-Tocopherol is an important chain-breaking antioxidant *in vivo* (Burton *et al.* 1982, 1983, 1985*a*; Ingold *et al.* 1987*b*) and in meats after slaughter (Marusich, 1980). Although it is generally accepted that α-tocopherol requirements of poultry are increased by consumption of thermally-oxidized lipids, there is little quantitative information on the effects of feeding such lipids on concentrations of α-tocopherol in tissues, and on the fatty acid composition and oxidative stability of tissues. Lin *et al.* (1989) detected lower α-tocopherol concentrations in dark and white muscle of broilers fed on standard all-mash diets supplemented with 55 g thermally-oxidized sunflower oil/kg compared with those fed on diets supplemented with 55 g fresh oil/kg. However, the α-tocopherol content of the oxidized-oil diet was considerably lower than that of the fresh-oil diet due to destruction of α-tocopherol in sunflower oil by heating, making the evaluation of that study difficult. The purpose of the present study was to investigate the effects of feeding diets containing fresh or heated sunflower oil, with or without α-tocopherol, on α-tocopherol concentrations and fatty acid composition of chick tissues, as well as on the stability of these tissues against Fe–ascorbate-induced lipid oxidation.

MATERIALS AND METHODS

Chemicals

All chemicals used were Analar grade obtained from British Drug House, Poole, Dorset, Sigma Chemical Co. Ltd., Poole, Dorset, and Rathburn Chemical Co. Ltd., Walkerburn, Peeblesshire. DL-α-tocopheryl acetate (Rovimix E) used in diets was purchased from Roche Products Ltd., Welwyn Garden City, Herts.

Animals and diets

Thirty-six 1-d-old female ISA Brown chicks were obtained from a commercial hatchery and housed in an environmentally controlled room. The temperature of the room was 32° during the first week, 28° during week 2 and 26° thereafter. A 12 h normal light–low light cycle was employed.

Chicks were randomly assigned to three groups and fed on glucose–soya-bean-based diets (Table 1), containing (g/kg): fresh sunflower oil (FSO) 40, heated sunflower oil (HSO) 40 or heated sunflower oil (40) supplemented with DL-α-tocopheryl acetate (HSE) to a similar α-tocopherol concentration as the fresh oil diet. Batches of sunflower oil were heated for 11 h at 120°. Air was constantly bubbled into the oil during heating. After determining the α-tocopherol concentration in both fresh and heated oil, the α-tocopherol level of the HSE diet was adjusted to that of the FSO diet. Butylated hydroxytoluene was added to the diets (via the oil) to a final dietary concentration of 0·05 g/kg, a level which protects linoleic acid and α-tocopherol for over 1 week at room temperature (Budowski *et al.* 1979). Diets were prepared weekly and stored at 4° during the experiment. As a check

Table 1. *Composition of diets fed to chicks (g/kg)**

Sunflower oil†	40·0
Soya-bean meal	520·0
Glucose monohydrate	383·6
Cellulose	10·0
DL-Methionine	1·40
Mineral mix‡	40·0
Vitamin mix§	5·00
Butylated hydroxytoluene	0·05

* Batches of diet were prepared weekly and stored at 4°.

† Fresh sunflower oil (FSO), heated sunflower oil (120° × 11 h with constant agitation; HSO), or heated sunflower oil supplemented with α-tocopheryl acetate (HSE).

‡ Supplying (mg/kg feed): $CaHPO_4$ 28 g, $CaCO_3$ 7 g, NaCl 3·5 g, $MnSO_4.4H_2O$ 488, $ZnCO_3$ 145, Ferric citrate 165, $CuSO_4.4H_2O$ 11·8, KI 2·35; $CoSO_4.8H_2O$ 1·45.

§ Supplying (mg/kg feed): retinol 3000 IU, cholecalciferol 200 IU, menadione sodium bisulphite 1, thiamine 3·6, riboflavin 7·2, calcium pantothenate 20, niacin 55, pyridoxine 6, biotin 0·2, folic acid 2·4, cyanocobalamin 0·02, choline chloride 1·3 g.

These amounts were premixed with 3·6 g glucose monohydrate.

on the accuracy of formulation, pooled samples of diet were stored at −20° and the α-tocopherol concentrations measured in triplicate at the end of the experiment. The diets were found to contain (mg α-tocopherol/kg): FSO 30·0 (SE 3·9), HSE 25·1 (SE 1·1), HSO 1·3 (SE 0·4). Peroxide values of typical batches of fresh and heated oils (measured in triplicate) were (mg peroxide-O/kg): 7·73 (SE 1·79) and 66·3 (SE 5·90), while the I values were 97·3 (SE 2·85) and 89·0 (SE 5·11) respectively. A small but significant decrease in the concentration of linoleic acid (18:2 *n*-6) was observed in sunflower oil after heating (Table 2).

Groups of chicks were housed in raised wire cages (1·2 × 1·0 × 0·8 m). Feed and water were provided *ad lib.* Body weights were determined twice weekly. After 32 d the chicks were fasted overnight, and killed by cervical dislocation. Blood was collected and plasma prepared by centrifugation at 1000 ***g*** for 25 min. Tissues were frozen in liquid N_2 and stored at −20° until required.

Analytical methods

α-Tocopherol was extracted from unsaponified plasma with hexane by the method of Shearer (1986). The hexane was evaporated with N_2 at 37° and the residue re-dissolved in 100 μl absolute alcohol. Tissues and oils were saponified by the method of Buttriss & Diplock (1984) before extraction of α-tocopherol. Tissues were homogenized in ice-cold KCl (11·5 g/l). Homogenate (1 ml) was added to 2 ml pyrogallol (10 g/l) in absolute alcohol, followed by 0·3 ml saturated KOH. The mixture was heated at 70° for 30 min, extracted once with 5 ml hexane–water (4:1, v/v), twice with hexane (2 ml), and centrifuged to separate the aqueous and organic layers. The hexane was evaporated under N_2 and the residue re-dissolved in 100 μl absolute alcohol. A similar procedure was performed for oils, except that samples of 20 mg oil were used. The α-tocopherol content of diets was determined after saponification by the method of Brubacher *et al.* (1985). Methanolic ascorbic acid (50 ml) and KOH (500 g/l; 10 ml) were added to finely ground samples of diet (10 g). The mixture was simmered on a steam bath under N_2 for 20 min, allowed to cool, and extracted three times with diethyl ether. The diethyl ether layer was collected using a separating funnel, removed by rotary evaporation, and the residue re-dissolved in absolute alcohol. α-Tocopherol was measured by reverse-phase HPLC using a Licrosorb RP18 (5 μm) column, a mobile phase of methanol–water (97:3, v/v) at 2·5 ml/min, and detection at 292 nm, as previously described (Sheehy *et al.* 1991). Recovery of α-tocopherol from plasma was determined by the addition of an internal standard, α-

Table 2. *Fatty acid composition (g/100 g total fatty acids) of diets containing fresh sunflower oil (FSO), heated, α-tocopheryl acetate-supplemented sunflower oil (HSE) or heated sunflower oil (HSO) fed to chicks**

(Mean values with their standard errors)

	FSO		HSE		HSO	
Fatty acid	Mean	SE	Mean	SE	Mean	SE
14:0	2·27	0·65	0·80	0·08	0·91	0·01
16:0	8·69	0·06	9·20	0·08	9·45	0·14
18:0	3·52	0·04	3·73	0·09	3·86	0·02
18:1	26·4	0·34	28·1	0·06	27·6	0·09
18:2	58·7[a]	0·30	57·6[b]	0·14	57·1[c]	0·07
18:3	0·39	0·01	0·38	0·01	0·37	0·02
Others		0·00	0·17	0·10	0·55	0·12

[a, b, c] Means with unlike superscript letters were significantly different ($P < 0·05$).
* For details of dietary composition, see Table 1.

tocopheryl acetate, to each sample before extraction. Because of the need for saponification, mean recoveries of α-tocopherol from diets, oils and tissues were estimated by spiking samples with graded amounts of α-tocopherol before saponification. Recovery was calculated from the slope ratio of the regression lines for observed and expected α-tocopherol concentration. Mean recoveries of α-tocopherol were always greater than 90%.

The concentrations of TBARS in plasma were determined by a spectrofluorometric method (Yagi, 1984). Tissue homogenates were assayed for TBARS before and after stimulation of lipid peroxidation with Fe–ascorbate by a modification of the method of Kornbrust & Mavis (1980). To a screw-capped glass tube was added 500 μl Tris–maleate buffer (80 mM, pH 7·4); 200 μl $FeSO_4 . 7H_2O$ (5 mM); 200 μl ascorbic acid solution (2 mM) and 100 μl tissue homogenate (100 mg/ml). Solutions were incubated for various lengths of time in a shaking water-bath at 37°. Following incubation the reaction was terminated and the TBARS concentration determined by the method of Beuge & Aust (1978). TBARS were expressed as nmol malondialdehyde (MDA)/mg tissue protein using $E_{MDA} = 1·56 \times 10^5$/M per cm. Protein was determined by the method of Lowry *et al.* (1951).

Neutral and polar lipids were extracted from 0·25–1·0 g tissue samples by the dry column method of Marmer & Maxwell (1981). BHT (0·05 g/l) was added to the extracting solvents. Following extraction the solvent was removed using a rotary evaporator and the lipids were re-dissolved in 5 ml hexane. Neutral lipids were converted to their methyl esters with methanolic BF_3 (Slover & Lanza, 1979), while those of polar lipids were esterified using 2 M-KOH in methanol (Maxwell & Marmer, 1983). Total lipids in diets were extracted by the method of Burton *et al.* (1985*b*). To prepare fatty acid methyl esters, 1 ml of the interesterification reagent (methanolic BF_3–benzene–methanol, 25:20:55, by vol.) was added and the samples heated for 30 min at 100°. Following addition of 1 ml water and 2 ml hexane, samples were shaken for 2 min, centrifuged and the hexane layer stored at −20°. Fatty acid methyl esters were analysed by GLC, using a Shimadzu gas–liquid chromatograph equipped with a Silar 10-C packed column. The oven temperature was held at 165° for 2 min, increased by 3° per min to 225°, held for 2 min, increased by 20° per min to a final temperature of 235° and held for 7 min. Injector and detector temperatures were 300°. Fatty acid methyl esters were identified by comparison of retention times with those of authentic standards.

Peroxide values of the fresh and heated oils were determined by the method of Lea (1946).

Statistical analysis

Statistical significance of the difference between means was determined by Student's *t* test, while linear regression was used to assess the relationship between α-tocopherol and TBARS concentrations. All statistical procedures used routines available in the Minitab Statistical Package (Ryan *et al.* 1986).

RESULTS

The effect of feeding diets containing fresh or heated sunflower oils on body-weight gain of chicks is shown in Fig. 1. Although feeding HSO reduced weight gain, mean body weights of the three groups did not differ significantly until day 32. At this point, however, mean body weights of chicks fed on HSO were significantly lower than those of chicks fed on FSO (−7·6%), or HSE (−8·2%).

Table 3 shows the effect of feeding chicks on HSO, HSE or FSO on the α-tocopherol concentrations of plasma and other tissues. High α-tocopherol concentrations were found in heart, liver, lung and spleen while low concentrations were found in thigh muscle, pancreas, brain and breast muscle. With the exception of brain, concentrations of α-tocopherol in tissues of chicks fed on FSO were significantly higher than those of chicks fed on HSE. In turn, the values for the latter group were significantly higher than those of the group fed on HSO. The concentrations of α-tocopherol in tissues of the group fed on HSE, expressed as a percentage of those of the group fed on FSO, are also shown in Table 3. Tocopherol concentrations in tissues of chicks fed on HSE ranged from 29·6 to 86·1 % of those of the FSO group, with the greatest depression in α-tocopherol concentration being evident in liver, heart and plasma. The relationship between plasma α-tocopherol concentration and the concentration of α-tocopherol in tissues was investigated by linear regression (Table 3). A statistically significant direct correlation was observed between plasma α-tocopherol concentration and the concentration of α-tocopherol in other tissues, the correlation coefficients ranging from 0·67 (plasma α-tocopherol *v.* pancreas α-tocopherol) to 0·94 (plasma α-tocopherol *v.* spleen α-tocopherol).

Chicks fed on HSO had significantly higher TBARS concentrations in plasma compared with both HSE and FSO groups (Fig. 2). However, values for the HSE and FSO groups did not differ significantly with respect to each other. The relationship between α-tocopherol and lipid peroxide concentrations in plasma is shown in Fig. 3. A statistically significant negative correlation was observed between the log of plasma α-tocopherol concentration and the concentration of TBARS in plasma.

Fig. 4 shows the effect of feeding HSO, HSE or FSO to chicks on TBARS concentrations in tissues, before and after stimulation of peroxidation with Fe–ascorbate. Before stimulation TBARS concentrations in tissues from the various groups were generally very similar. Peroxidation was stimulated by incubating tissue homogenates with Fe–ascorbate at 37° for various lengths of time. With the exception of brain, tissues from chicks fed on HSO peroxidized more rapidly than those of the other two groups, while the rate of peroxidation of tissue homogenates from chicks fed on HSE was generally faster than those of the group fed on FSO.

The effect of feeding HSO, HSE or FSO on the fatty acid composition of chick tissues was also investigated. No significant differences in fatty acid composition of neutral or polar lipids of breast muscle and brain were observed between groups (values not shown). Slight changes were observed in the proportions of 18:0, 18:1, 20:5 and 22:0 in liver neutral lipids, and in the content of 18:1 in polar lipids of chicks fed on different diets. However, these changes were small and no definite trends were discernable (values not

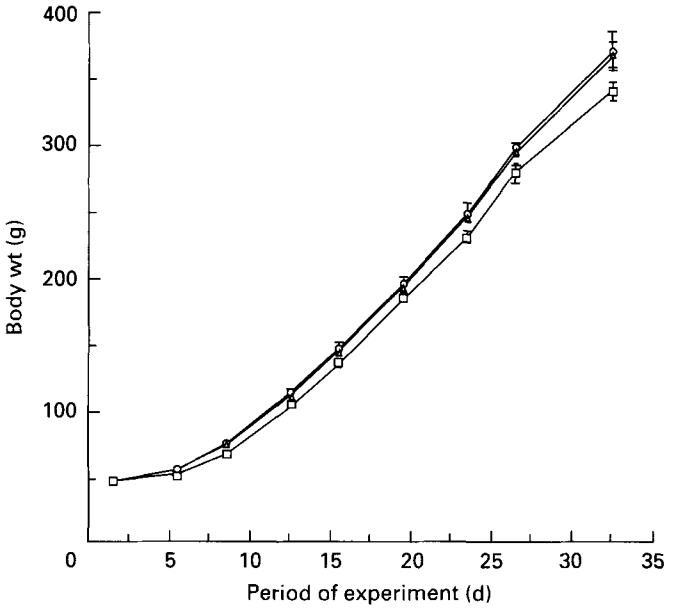

Fig. 1. Body weights of chicks fed on diets containing fresh sunflower oil (FSO; △), heated sunflower oil (HSO; □), or heated, α-tocopheryl acetate-supplemented sunflower oil (HSE; ○). For details of diets and procedures, see Tables 1 and 2 and pp. 54–55. Points are means with their standard errors represented by vertical bars. Mean value for HSO was significantly different from that for FSO and HSE: * $P < 0.05$.

Table 3. *Effect of feeding heated sunflower oil (HSO), heated, α-tocopheryl acetate-supplemented sunflower oil (HSE) or fresh sunflower oil (FSO) on α-tocopherol concentrations in chicken tissues**

(Mean values with their standard errors)

	α-Tocopherol (μg/ml plasma or ng/mg protein)							Relationship between plasma and tissue α-tocopherol concentrations	
	HSE		HSE		FSO		HSE:FSO (%)		
Tissue	Mean	SE	Mean	SE	Mean	SE	Mean	(*r*)	Statistical significance of difference: ($P <$)
Plasma	0·83[a]	0·08	6·65[b]	1·01	16·6[c]	1·40	40·1	—	—
Liver	3·00[a]	0·96	40·0[b]	2·20	135[c]	25·9	29·6	0·765	0·001
Heart	10·5[a]	1·80	106[b]	5·50	326[c]	54·1	32·5	0·926	0·001
Thigh muscle	6·07[a]	0·79	33·4[b]	4·30	58·1[c]	4·30	57·5	0·934	0·001
Breast muscle	4·59[a]	0·44	19·1[b]	2·12	28·8[c]	1·80	66·3	0·921	0·001
Lung	14·0[a]	6·20	64·6[b]	8·80	147[c]	32·2	43·9	0·771	0·001
Pancreas	3·06[a]	0·73	26·1[b]	4·30	40·2[c]	3·40	64·9	0·671	0·005
Spleen	4·53[a]	0·65	61·0[b]	4·11	121[c]	10·3	50·4	0·937	0·001
Brain	15·5[a]	1·51	27·9[b]	2·91	32·4[b]	3·30	86·1	0·753	0·005

[a, b, c] Mean values for α-tocopherol concentrations in horizontal rows with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Tables 1 and 2 and pp. 54–57.

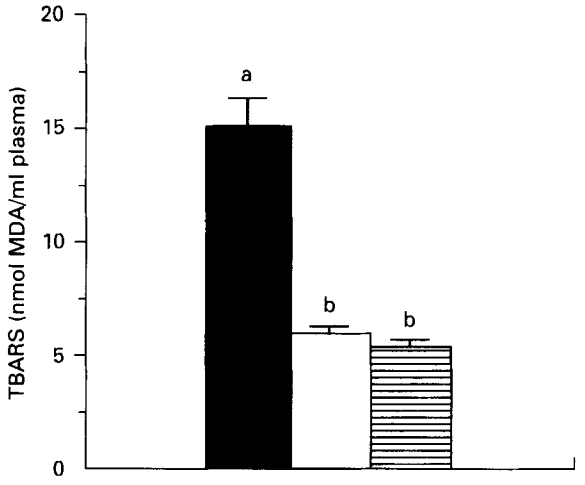

Fig. 2. Concentrations of thiobarbituric acid-reacting substances (TBARS) in plasma of chicks fed on heated sunflower oil (HSO; ■), heated, α-tocopheryl acetate-supplemented sunflower oil (HSE; □) or fresh sunflower oil (FSO; ⊟). For details of diets and procedures, see Tables 1 and 2 and pp. 54–57. Points are means with their standard errors represented by vertical bars. [a,b], Mean values with unlike superscript letters were significantly different ($P < 0.05$). MDA, malondialdehyde.

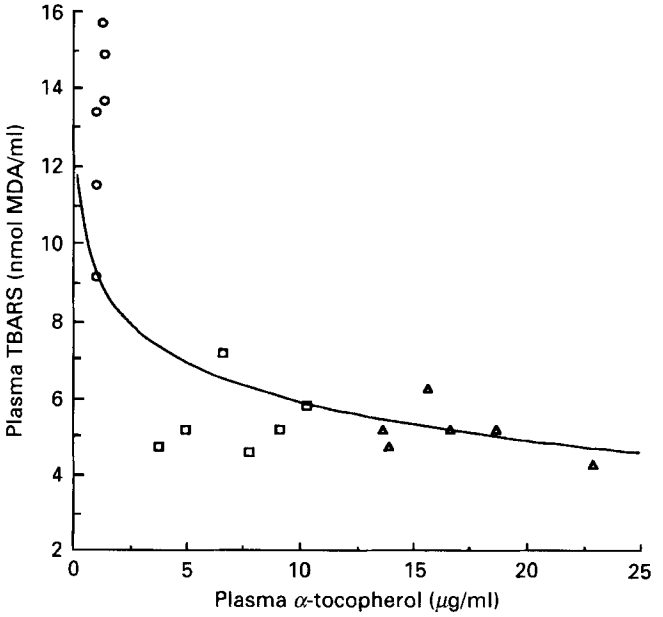

Fig. 3. Relationship between plasma concentrations of α-tocopherol and thiobarbituric acid-reacting substances (TBARS) of chicks fed on diets containing heated sunflower oil (HSO; ○), heated, α-tocopheryl acetate-supplemented sunflower oil (HSO; □), or fresh sunflower oil (FSO; △) ($r = -0.851$, $P < 0.001$). For details of diets and procedures, see Tables 1 and 2 and pp. 54–57. MDA, malondialdehyde.

shown). Slight, but not significant, increases in the proportions of 18:2, 20:4 and total polyunsaturated fatty acids were observed in neutral lipids of heart and lung, as well as a decrease in monounsaturates (16:1 and 18:1) in heart neutral lipids, after feeding FSO (Tables 4 and 5). No significant differences were observed in the fatty acid composition of

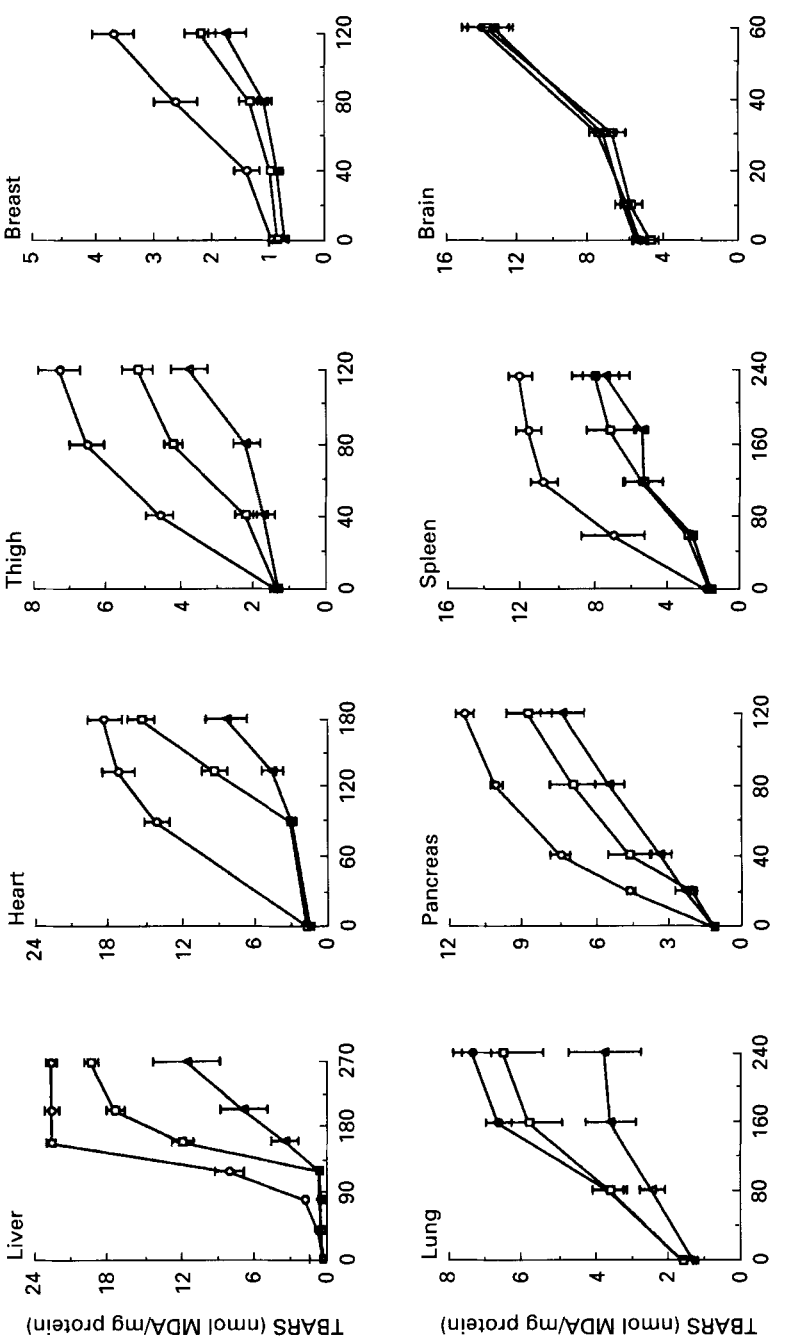

Fig. 4. Iron–ascorbate-induced lipid peroxidation in tissues of chick fed on diets containing heated sunflower oil (HSO; ○), heated, α-tocopheryl acetate-supplemented sunflower oil (HSE; □), or fresh sunflower oil (FSO, △). Points are means with their standard errors represented by vertical bars. For details of diets and procedures, see Tables 1 and 2 and pp. 54–57. MDA, malondialdehyde.

Table 4. *Fatty acid composition (g/100 g total fatty acids) of neutral and polar lipid fractions of heart of chicks fed on diets containing heated sunflower oil (HSO), heated, α-tocopheryl acetate-supplemented sunflower oil (HSE) or fresh sunflower oil (FSO)**

(Mean values with their standard errors for six determinations)

	Neutral lipids						Polar lipids					
	HSO		HSE		FSO		HSO		HSE		FSO	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16·0	18·7	0·78	20·2	1·29	19·5	1·39	20·5	0·27	20·7	0·47	19·7	0·46
16·1	2·59	0·43	3·33	0·13	1·96	0·32	1·10	0·42	1·60	0·33	1·45	0·32
18·0	11·3	1·40	10·5	0·53	9·97	22·8	21·0	2·52	18·7	2·05	22·6	2·71
18·1	27·2	2·06	27·0	0·61	22·8	1·77	16·1	3·00	19·4	2·19	15·5	2·47
18·2	32·6	1·13	31·3	1·12	35·7	1·62	26·8	1·64	26·1	1·61	24·3	2·19
20·0	0·05	0·05	0·04	0·04	0·17	0·14	0·00		0·00		0·00	
18·3	1·04	0·11	0·88	0·02	0·76	0·17	0·46	0·16	0·62	0·08	0·45	0·07
22·0	0·15	0·12	0·48	0·21	0·03	0·03	0·75	0·11	0·68	0·11	0·63	0·12
20·4	5·33	1·77	5·67	1·30	8·69	2·55	12·1	2·11	10·5	1·75	10·9	1·81
20·5	0·46	0·12	0·26	0·04	0·32	0·09	0·19	0·06	0·15	0·06	0·24	0·07
22·6	0·56	0·28	0·16	0·09	0·13	0·05	0·80	0·35	0·41	0·27	0·63	0·32
PUFA	40·0	1·90	38·3	1·49	45·6	2·23	40·4	1·03	38·4	0·62	36·5	0·93

PUFA, polyunsaturated fatty acids.

* For details of diets and procedures, see Tables 1 and 2 and pp. 54–57.

Table 5. *Fatty acid composition (g/100 g total fatty acids) of neutral and polar lipid fractions of lung of chicks fed on diets containing heated sunflower oil (HSO), heated, α-tocopheryl acetate-supplemented sunflower oil (HSE) or fresh sunflower oil (FSO)**

(Mean values with their standard errors for six determinations)

	Neutral lipids						Polar lipids					
	HSO		HSE		FSO		HSO		HSE		FSO	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
16·0	24·7^{a}	0·85	22·6$^{a\,b}$	0·67	21·2^{b}	0·72	37·5	1·66	36·6	2·08	36·0	1·64
16·1	1·47	0·20	2·33	0·56	1·70	0·30	1·22	0·11	1·68	0·21	1·26	0·19
18·0	14·2^{a}	0·96	10·6^{b}	1·23	11·8$^{a\,b}$	1·67	9·05	0·23	8·60	0·23	9·79	0·63
18·1	24·0	1·55	24·6	2·30	25·3	2·14	12·0	0·58	14·9	1·78	13·7	1·42
18·2	24·4	1·09	24·9	1·73	28·3	2·04	10·1	0·70	12·9	1·74	13·0	2·37
20·0	0·90	0·18	1·27	0·39	0·92	0·41	0·00		0·00		0·10	0·03
18·3	0·68	0·04	3·76	3·10	0·51	0·23	0·00		0·16	0·16	0·30	0·10
22·0	0·47	0·26	0·76	0·36	0·53	0·27	0·00		0·32	0·12	0·76	0·25
20·4	7·89	1·18	7·71	2·52	9·47	2·42	11·6	0·53	10·8	0·74	11·5	1·04
20·5	0·41	0·12	0·28	0·15	0·00		0·00		0·00		0·34	0·11
U	0·77	0·26	1·24	0·51	0·25	0·17	18·6^{a}	0·94	14·0^{b}	1·18	13·3^{b}	2·02
PUFA	34·2^{b}	1·18	37·9$^{a\,b}$	2·38	38·6^{a}	0·70	40·2	1·51	37·9	0·80	38·4	1·22

$^{a,\,b}$ For neutral or polar lipids, mean values with unlike superscript letters were significantly different ($P < 0·05$).

U, unknown; PUFA, polyunsaturated fatty acids.

* For details of diets and procedures, see Tables 1 and 2 and pp. 54–57.

heart polar lipids between groups. In lung polar lipids an unknown fatty acid which eluted slightly earlier than 22:6 was significantly increased in chicks fed on HSO compared with the other groups.

DISCUSSION

In the present study a significant growth depression was observed when chicks were fed on HSO, relative to those fed on FSO or HSE. There are several reports in the literature showing that feeding heat-abused fats or oils depresses animal growth (Calabotta & Shermer, 1985; Lin *et al.* 1989). Because of the complex nature of the products formed when fats are oxidized, growth depression could be due to a number of factors, including differences in feed consumption, digestibility and protein quality. Lin *et al.* (1989) reported a 4·2% decrease in body weight of broilers fed on oxidized sunflower oil diets containing no added tocopherol, compared with that of birds fed on fresh sunflower oil diets. Feed consumption did not differ significantly between groups. These authors suggested that the decreased body weight could be partly due to a toxic effect of lipid oxidation products, and partly to a decrease in the biological value of the heated oil, destruction of fat-soluble vitamins and carotenoids, and lower protein quality due to reaction of dietary protein with oxidation products. In the present study feed consumption was not measured because the birds generally scattered a considerable amount of the feed and, thus, it is not clear whether the palatability of HSO and HSE was similar to that of FSO. However, the reduced weight gain in chicks fed on HSO was probably a combined effect of oxidation products and α-tocopherol deficiency since the feeding of oxidized oil with adequate α-tocopherol did not depress growth significantly (HSE *v.* FSO), while in a previous study α-tocopherol deficiency did not depress weight gain (Sheehy *et al.* 1991).

Chicks fed on HSO had extremely low plasma and tissue α-tocopherol concentrations. This finding would be expected since the diet contained only 1·3 mg α-tocopherol/kg. Of greater interest, however, was the observation that the α-tocopherol concentrations of plasma and tissues (except brain) of the HSE group were significantly lower than those of chicks fed on FSO. The values ranged from 29·6 to 86·1% of the FSO values. The reduction was more pronounced in tissues rich in α-tocopherol, possibly because of the relatively fast turnover of the vitamin in these tissues (Ingold *et al.* 1987*a*). The studies of Lin *et al* (1989) showed that α-tocopherol concentrations in dark and white muscle of broilers fed on oxidized sunflower oil were significantly lower than those of birds fed on fresh oil. However, in their study no attempt was made to correct for the tocopherol destroyed by heating, so the α-tocopherol content of the oxidized-oil diet was considerably lower than that of the fresh-oil diet. Thus, their results may simply have been due to a lower intake of α-tocopherol. Although HSE and FSO in the present study were formulated to contain the same concentration of α-tocopherol, the pooled sample of HSE analysed at the end of the experiment had a slightly lower α-tocopherol concentration than the pooled FSO (25·1 (SE 1·1) *v.* 30·0 (SE 3·9) mg/kg, or a mean value of 84%). The difference was probably due to problems in sampling or deterioration of α-tocopherol during storage, rather than because of errors in the formulation of the diet, since the α-tocopherol levels in the fresh and heated oils were measured before the diets were prepared. However, even if this difference existed during the entire feeding study it would be unlikely to account for the large depression in α-tocopherol concentration observed in some HSE tissues, especially liver, heart and plasma, in which tocopherol levels were only about 30–40% of the FSO values. The reduced levels could be due to the destruction of α-tocopherol in the gastrointestinal tract by free radicals present in the heated oil, including those produced by Fe- or Cu-catalysed breakdown of hydroperoxides. Alternatively, the hydrolysis of α-tocopheryl acetate in the

intestine may be impaired by lipid oxidation products. In either case the amount of α-tocopherol available for absorption would be reduced. A third possibility could be that α-tocopherol in plasma is oxidized by peroxyl radicals or lipid hydroperoxides absorbed from the diet. However, the available evidence indicates that lipid hydroperoxides are poorly absorbed, while peroxyl radicals would be expected to react with other unsaturated fatty acids either in the gastrointestinal tract or in the enterocyte, rather than being transferred to the lymph or plasma in that form. Thus, the oxidation of plasma α-tocopherol by hydroperoxides or peroxyl radicals from the diet should not be a major factor, although it may be speculated that the absorption and subsequent breakdown of even small quantities of lipid hydroperoxides could be sufficient to initiate free-radical chain reactions in plasma. Furthermore, because of the large number of oxidation products present in thermally-abused oils, other compounds could play a role in degrading plasma α-tocopherol. Izaki *et al.* (1984) reported that feeding oxidized rapeseed oil at graded levels of deterioration (in diets containing 90 mg α-tocopheryl acetate/kg) to rats decreased serum and liver α-tocopherol concentrations and increased liver TBARS in proportion to the degree of oxidative damage of the oil. These authors speculated that α-tocopherol is degraded by oxidation products in the intestinal lumen or in liver membranes, and suggested that unsaturated keto compounds formed in heated oils, such as 12-keto oleate, may be absorbed and cause lipid peroxidation *in vivo*. Although the quantities of oxidized lipids consumed by the chicks in the present study are much higher than those likely to be consumed in a normal diet, it is nevertheless of considerable interest that plasma and tissue α-tocopherol concentrations were negatively influenced by feeding oxidized dietary lipids, given that α-tocopherol is the major lipid-soluble chain-breaking antioxidant in both plasma (Burton *et al.* 1982; Ingold *et al.* 1987*b*; Niki *et al.* 1988) and other tissues (Cheeseman *et al.* 1986, 1988), and the current widespread interest in the role of lipid peroxidation and free-radical defence mechanisms in the aetiology and prevention of various chronic disease states.

A significant inverse correlation was observed between the log of plasma α-tocopherol concentration and the concentration of TBARS in plasma (Fig. 3). There is some evidence that malondialdehyde absorption occurs in rats (Draper *et al.* 1984), but evidence is lacking on this point in chicks. It would be expected that if chicks absorb malondialdehyde and other lipid oxidation products, then the concentration of TBARS in plasma of both the HSO and HSE groups should be high, since both diets contained peroxidized lipids. However, this effect was not observed in the present study. Plasma TBARS concentrations for the HSE group, although slightly higher, did not differ significantly from those of the group fed on FSO (Fig. 2). This finding suggests that the significant elevation in TBARS concentrations in the plasma of chicks fed on HSO resulted from peroxidation of plasma lipids, rather than from absorption of malondialdehyde and other TBA-reactive substances from the gastrointestinal tract, and were due to the lower concentrations of α-tocopherol in plasma. The observed relationship between plasma α-tocopherol and TBARS is in agreement with previous results from this laboratory in chicks fed on various levels of α-tocopherol. A similar relationship between plasma α-tocopherol and plasma malondialdehyde concentrations has been observed in trout (Frigg *et al.* 1990).

Our finding that plasma and tissue α-tocopherol concentrations were significantly correlated (Table 3) extends our previous observation that plasma α-tocopherol concentration is a good indicator of tissue α-tocopherol status in the young chick (Sheehy *et al.* 1991) and supports that of Whitacre *et al.* (1987) who observed a significant direct correlation between plasma α-tocopherol concentration and the concentration of α-tocopherol in liver, heart and pancreas of chicks fed on various concentrations of the vitamin.

The increased susceptibility of tissues (except brain) of chicks fed on HSO and HSE to Fe–ascorbate-induced lipid oxidation (Fig. 4) was probably due to the lower levels of α-tocopherol in these tissues, since susceptibility to lipid oxidation is markedly influenced by tissue α-tocopherol concentration (Franco & Jenkinson, 1986; Hu *et al.* 1989; Leibovitz *et al.* 1990). Because of the important role played by α-tocopherol in preventing oxidative deterioration of chicken muscle during refrigerated and frozen storage (Marusich *et al.* 1975), the feeding of diets containing thermally-abused oils to chicks may reduce the storage stability of chicken meat and meat products, particularly those containing pre-cooked or comminuted meat.

The effect of feeding diets containing heated or fresh sunflower oil on the fatty acid composition of chick tissues was also investigated in the present study (Tables 4 and 5). In general, the differences observed between groups were rather small, particularly when the slight differences in fatty acid composition of the diets are taken into account (Table 2). Because of the dependence of tissue fatty acid composition on the composition of dietary lipids, it is likely that tissue fatty acid compositions of chicks fed on HSO and HSE would differ more substantially from those of chicks fed on FSO if the heating procedure had destroyed a greater proportion of linoleic acid in the sunflower oil.

In conclusion, the feeding of HSO to chicks significantly reduced body weight gain compared with feeding FSO or HSE. The presence of oxidation products in diets was associated with a significant reduction in α-tocopherol concentrations in plasma and other tissues. Plasma α-tocopherol concentrations were positively correlated with those in other tissues, while plasma TBARS were negatively correlated with log plasma α-tocopherol. Generally, susceptibility of tissues to Fe–ascorbate-induced lipid oxidation was increased by feeding HSO.

Supplementing HSO with α-tocopheryl acetate (HSE) reduced susceptibility to varying degrees, depending on the tissue. Minor changes in the fatty acid composition of liver, heart and lung were observed. The results suggest that chronic ingestion of thermally-oxidized lipids may compromise the activity of free-radical defence mechanisms *in vivo* by depleting α-tocopherol in the gastrointestinal tract, or possibly in plasma and other tissues.

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