Effects of dietary fish oil on the composition and stability of turkey depot fat

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1. Beef fat (2·5%) and anchovy oil (2·5%; 2·5% plus 0·02% ethoxyquin; 5%) were incorporated into isocaloric cereal-based diets A, B, C and D and given to turkeys from 2 to 10 weeks of age. Diets A, B, and C contained 13 and diet D 26 i.u. α-tocopheryl acetate/kg. The lipids of diets B and D, which contained fish oil without ethoxyquin, autoxidized in the feeding troughs, but not seriously in the brief period of exposure permitted. 2. The birds all remained healthy and grew well, the only nutritional effects of the fish oil being a depressed storage of vitamin A in the liver at both levels of feeding (prevented by ethoxyquin) and a slightly adverse effect, at the higher level only, on food conversion ratio. 3. The skin fats of the birds given fish oil contained seven major and two minor fatty acids, derived from the fish oil, which were not present in the skin of the control group given beef fat, as well as one major fish oil acid present in much greater concentration in the birds given fish oil than in the controls. All these acids were present in the skin fats at about half or two-thirds of their concentrations in the dietary lipid, except for acid 22:5, which reached two or three times this level. 4. The stabilities towards autoxidation of the skin fats decreased in the order A > C > B > D, as their content of polyunsaturated fatty acids increased, but the greater stability of group C as compared with B was probably due, in the main, to the higher tocopherol content of the skin fat of the birds on the ethoxyquin-containing diet. Fishy flavours which developed on cooking showed a similar relationship to diet.

Though adequate supplies of polyunsaturated fatty acids (PUFA) are necessary for the animal body to produce lipids of proper biological function, adverse effects can ensue if such acids are present in the diet in concentrations that are high in relation to those of vitamin E and other substances of related protective activity. In chicks, when the diet is deficient in vitamin E, polyunsaturated acids of the linoleic–arachidonic series cause encephalomalacia, and Dam & Søndergaard (1964) have shown that approximately 0·8 mg D-α-tocopherol is required to balance each g of linoleic acid present in the diet. Neither selenium nor sulphur-containing amino acids protect against this sign of vitamin E deficiency, though certain synthetic antioxidants do so, e.g. ethoxyquin (EMQ; 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline). On diets low in selenium and vitamin E (or other biologically active antioxidant) the giving of PUFA causes or greatly increases the severity of exudative diathesis. On diets low in sulphur amino acids and vitamin E, muscular dystrophy develops, though this sign is less dependent on the fat content of the diet and some evidence has recently been produced disputing any direct relation between muscular dystrophy in the chick and increased susceptibility of the tissue lipids to oxidation (Desai, Calvert & Scott, 1964). Nevertheless, Calvert, Desai & Scott (1964) found that the amount of vitamin E required to prevent muscular dystrophy in chicks increased with the amount of linoleic acid ingested up to 0·5% (though thereafter no further increase in vitamin E was required up to 2·5% linoleate), and Miller, Leong, Knobl &

Gruger (1965) have demonstrated that sufficiently high levels of highly unsaturated fish oil esters or glycerides will cause both these vitamin E deficiency syndromes in the presence of normally effective levels of protectants. Miller *et al.* (1965) assess the relative protective potencies of selenium, DL-α-tocopheryl acetate and EMQ for this purpose as approximately 1:500:3750, on a weight basis.

It may be that PUFA increases the requirement for vitamin E and related protective substances partly because of increased destruction of the vitamin by free radicals produced during autoxidation of the unsaturated fatty acids, either before or during absorption. Probably more important, however, is the resulting increased deposition of these readily peroxidizable fatty acids in important lipid structures, such as the membranes of mitochondria and lysosomes, where their biological function could be damaged by peroxidation under the influence of catalysts now believed to consist of both haem proteins and inorganic iron (Wills, 1966).

Another important consequence of giving fats containing high levels of PUFA to non-ruminant meat animals is an increased tendency for the development of 'fishy' odours and flavours on cooking: at lower dietary levels of PUFA 'off' flavours may result which are not recognizable as 'fishy'. Taints of this kind probably arise from peroxidation of the increased quantities of oxidatively unstable fatty acids present in the tissues, with subsequent fission to volatile, malodorous fragments.

In a previous experiment (Lea, Parr & Carpenter, 1960) we failed to detect any fishy taint in the eggs or flesh of hens on diets containing 8% of fresh or stored herring meal, equivalent to a dietary content of nearly 1·4% of chloroform–methanol extractable fish lipid or approximately 1·0% of ether or light petroleum extract. More recently (Lea, Parr, L'Estrange & Carpenter, 1966) we have found that the presence of fish oil (in fresh fish meal), at a level of 2·2% of the diet, caused serious tainting of the flesh of roast turkeys; the same amount of oil oxidatively polymerized by storage of the meal before feeding caused only negligible tainting. Stabilization of the oil in fish meal by the use of antioxidants is therefore likely to have an adverse effect on the flavour of the meat produced, though the protein of stabilized meal may be better nutritionally because the oxidized oil can cause damage to the protein (Lea *et al.* 1960; Carpenter, Morgan, Lea & Parr, 1962). At levels likely to be met in practice the oxidized oil of stored fish meal is not itself directly toxic to chicks (Carpenter, Lea & Parr, 1963).

In a recent review of the rather contradictory literature on the subject of tainting by fish oil Fry, van Walleghem, Waldroup & Harms (1965) have concluded that 'off' flavours most commonly appear when the fish oil content of the diet reaches 1·5–2·0%, indicating a necessity to keep it below 1·0–1·5%. There have been some reports that the addition of extra tocopherol or of synthetic antioxidants to diets containing fish oil or meal reduces tainting of the flesh of pigs or poultry to a limited degree, but in other instances no apparent improvement has resulted.

Since the physiological and organoleptic consequences of feeding fish oil to poultry presumably stem alike from the transference of some of the characteristic PUFA (with or without modification) from the fish oil to the body tissues, it was of interest to apply the improved techniques now available for lipid analysis to trace this trans-

ference into the storage depot fat and into the lipids of the muscular tissue. The present paper, therefore, compares diets containing anchovy oil at the 2·5% (with and without antioxidant) and 5% levels with a control containing 2·5% beef fat, for their effects on the growth of turkeys, and on the chemical composition, oxidative stability and palatability of the skin fat (mainly triglyceride). In a subsequent paper the detailed composition of the fractionated lipids of the breast and leg muscle will be presented, to establish the extent to which individual PUFA of the fish oil enter individual lipids of the muscle.

EXPERIMENTAL

Thirty-six 1-day-old female turkey poults, of a strain (10/20 Broad Breasted White) of fast-maturing birds specially suited for slaughter at 5–6 lb weight, were obtained from Norfolk Manor Farms Ltd, Great Witchingham Hall, Norwich. For the first 2 weeks the birds were fed on a 'young bird diet' which, with the exception of a slightly higher protein content (26·3% *v.* 22·1%) was the same as the diet given subsequently to control group A. The birds were then weighed and eight randomized groups of four birds each selected to minimize differences between group weights. Four sets of two groups each were then given diets A, B, C and D respectively for a further 8 weeks. After the 2nd week on the experimental diets, when the birds were seen to be tolerating the diets well, one from each cage was discarded, leaving six birds on each diet. Feeding was *ad lib.* but residual food was removed from the troughs every 24 h and discarded, to minimize autoxidative changes in the lipids.

Diets

Each of the four diets contained 92 parts of a common basal mixture supplying the necessary growth factors in an economically feasible form. To this was added beef fat or fish oil and maize starch or cellulose powder, as indicated below, to adjust the four diets to the same calorific value.

Diet A: (beef fat control) 92 parts basal diet, 2·5 parts beef fat and 5·5 parts maize starch.

Diet B: 92 parts basal diet, 2·5 parts anchovy oil and 5·5 parts maize starch.

Diet C: as diet B, but with the addition of 0·02% EMQ.

Diet D: 92 parts basal diet, 5·0 parts anchovy oil and 3·0 parts cellulose powder (Whatman Standard Grade). The vitamin E content of this diet was raised by doubling the amount of Rovimix E (Roche Products Ltd) added.

Immediately after mixing, all four diets were packed under nitrogen in A $2\frac{1}{2}$ cans, the end seams of which were coated with a low-melting alloy (Wood's metal) to ensure complete gas-tightness. Storage was at $-10°$ until a few hours before opening for use.

Basal mixture. The 92 parts basal diet contributed: ground wheat 28, finely ground barley 28, extracted soya-bean meal 17, dried unextracted yeast 5, casein 8, sterilized steamed bone flour 2 and mineral, vitamin, antibiotic premix (in bone flour) 4. The premix, which was similar to that used in a previous experiment (Lea *et al.* 1966) with the addition of vitamin B_{12} (0·02 ppm of the final diet), contributed to the diet

7700 i.u. vitamin A/kg and 13 i.u. vitamin E/kg as α-tocopheryl acetate (26 i.u./kg for diet D).

Fats used. Beef fat was rendered from fresh beef adipose tissue. The anchovy oil, of Peruvian origin, in solution in light petroleum, was passed through a column of activated silicic acid and alumina to remove autoxidation products and other impurities, followed by distillation and deodorization with steam under reduced pressure to remove the solvent. After removal of a sample for fatty acid analysis by gas chromatography both fats were packed in sealed cans under nitrogen and stored at −80° until required.

Oxidation in the feeding troughs. Spare troughs half filled with diet were held under conditions similar to those in which the birds were feeding, and representative samples were removed daily to test for oxidation. The lipid from each sample was extracted by shaking with chloroform for 0·5 h, after which the peroxide value (pv) was determined on the extracted fat by the method of Lea (1952).

Performance of the birds

Weight increase and food consumption were measured throughout the experiment, and after the birds were killed their livers were weighed and analysed for vitamin A content (Lea, Parr, L'Estrange & Carpenter, 1964).

Analysis of the lipids

Extraction of the fat. Since practically no abdominal fatty tissue had been laid down, the skins were examined as the main fat storage depot. For this purpose the skins of each group were combined, cut up with scissors and mixed, and a representative sample was freeze-dried. The fat was then extracted from the dry tissue with peroxide-free ether, in darkness under nitrogen in an apparatus of the hot extraction type, and the solvent removed at low temperature under reduced pressure.

Conversion to methyl esters and analysis by gas chromatography. The fat (1 g) was refluxed in 400 ml dry 5% (w/v) sulphuric acid–methanol, with the addition of 25 ml benzene to ensure solution, for 4 h under nitrogen. The methyl esters were washed and dried by the method of Bowyer, Leat, Howard & Gresham (1963). After removal of a sample for determination of the iodine value a 10% (w/v) solution of the methyl esters in pure isooctane containing 5 mg % BHT (2,6-di-tert.butyl-4-methyl-phenol) was prepared for gas chromatography.

Hydrogenation. To assist identification of the very complex mixture of fatty acids present in the anchovy oil a sample (0·2 g) of the methyl esters from this oil was hydrogenated in methanol solution for 16 h at 37° with Adam's platinum oxide catalyst (50 mg), and the recovered saturated esters were subjected again to gas chromatographic analysis.

Thin layer chromatography. As a further aid to the identification of individual fatty acids the anchovy oil methyl esters were fractionated according to degree of unsaturation on a 20 × 20 cm 20% silver nitrate–silica gel H plate, using a modification of the method of Kenney, Komanowsky & Wrigley (1965). The sample of methyl esters (80–100 μl of the 10% solution in isooctane) was applied as a narrow band 2 cm from the bottom of the plate, and developed in a constant temperature room at 1°

with hexane-benzene (20:80, v/v) containing, per 100 ml, 10 mg BHQ (butylhydroquinone, Neudoerffer & Lea, 1966), which was found to be the most suitable solvent. We preferred to make the separated ester bands visible by spraying with rhodamine–fluorescein (rhodamine 100 mg and dichlorofluorescein 32 mg dissolved in ether 150 ml, ethanol 70 ml and water 17 ml), rather than with water as used by Kenney *et al.* (1965). After elution of the four separated fractions from the support with 3×5 ml light petroleum the solvent was distilled off and the residue redissolved in 0·2 ml isooctane containing 5 mg BHQ/100 ml for gas chromatographic analysis.

Gas chromatographic analysis. Analysis of the fatty acid methyl esters was carried out on a Pye Series 104, model 24 (dual column) flame ionization gas chromatograph, but only one of the columns was used. The 5 ft glass column (purchased from W. G. Pye and Co. Ltd, Cambridge) was packed with 100–120 mesh Celite, carrying 10% of polyethylene glycol adipate (PEGA) as liquid phase, and the temperature was programmed at 4°/min from injection at 150° to 200°, after which the analysis was completed isothermally at 200°. The detector temperature was held at 220° throughout, in a specially constructed compartment, to maintain uniformity of response. Argon at a flow rate of 40 ml/min was used as carrier gas and a 1:1 mixture of hydrogen and nitrogen supplied the flame. The voltage applied to the jet of the flame detector was increased from 45 to 90 V, to extend its linear dynamic range. With a sample injection of 1·5 μl of a 10% solution of the esters in isooctane the attenuation of the amplifier sensitivity was varied between 2000 and 50000 times (i.e. full-scale deflection varied from 2×10^{-9} to 5×10^{-8} A) and the chart speed was usually 30 in/h.

For calibration, mixtures of methyl myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidonate, eicosapentaenoate and docosahexaenoate (obtained from the Hormel Foundation, Austin, Minn. and the National Institutes of Health, Bethesda 14, Md) were prepared and assayed repeatedly. The last three of these esters were less pure (about 95%) than the others (> 99%) and were themselves assayed by gas–liquid chromatography (GLC), so that appropriate corrections could be made to the compositions of the mixtures containing them.

Tocopherol determination. Tocopherol (presumed to be mainly α- because of less efficient absorption and deposition of the other homologues) was determined on portions of the ether extracts of the skin by a slightly modified version of the method of Erickson & Dunkley (1964) using 4,7-diphenyl-1,10-phenanthroline. Replacement of the 0·1 M-orthophosphate specified by a 0·2 M solution was found to give more complete protection against photoreduction of residual ferric iron, and elution with 20 ml solvent, in place of the 10 ml suggested, was found to be necessary to achieve complete recovery of added tocopherol. A standard reference curve was constructed for hexane:benzene (2:3, v/v) solutions of pure DL-α-tocopherol.

Stability of the depot fat. For comparison of the stabilities of the skin fats towards autoxidation 0·2 ml portions were pipetted into a series of small glass cups and held at 60°, with determination of the peroxide value (pv) at suitable intervals, as previously described (Lea, 1960), but omitting the peroxide 'starter'.

RESULTS

Oxidation in the feeding troughs. The rates of peroxidation of the lipids of the four diets when stored in the feeding troughs under the conditions of the 1st week of the experiment (27° and bright fluorescent lighting) are shown in Fig. 1. Thereafter the temperature at the feeding troughs (which were remote from the infrared heating lamps) was reduced to 20°, from which it fell gradually to 15° at the conclusion of the experiment. The illumination was also much less bright than during the 1st week.

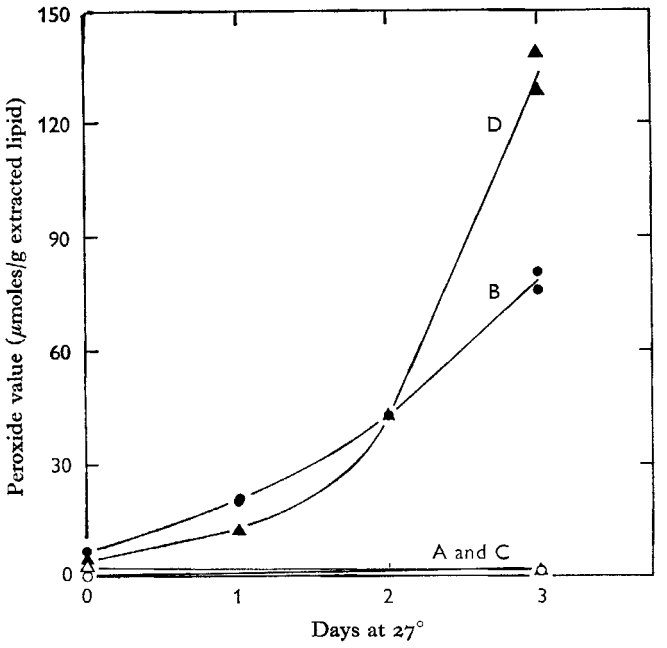

Fig. 1. Peroxidation of dietary lipids in the feeding troughs of turkeys. Diet A, 2·5% beef fat; diet B, 2·5% anchovy oil; diet C, 2·5% anchovy oil+EMQ; diet D, 5·0% anchovy oil.

Table 1. *Response of the turkeys to the experimental diets containing different fats*

Diet	Live-wt gain 2–10 weeks (g/poult)	Food conversion ratio (food consumed/wt gained)	Liver wt (as % body-wt)	Vitamin A reserves (i.u./liver)
A (2·5% beef fat, control)	2516	2·38	1·49	7060
B (2·5% anchovy oil)	2574	2·43	1·50	5680
C (as B, +0·02% EMQ)	2564	2·41	1·50	7280
D (5·0% anchovy oil)	2552	2·58	1·59	5550
Standard error of treatment means*	±38	±0·041	±0·055	±248

* In the analysis of variance the food conversion and the vitamin A content of the livers were significantly affected by treatment. In the former, diet D was just significantly higher than the other three ($P < 0·05$); in the latter, diets A and C were higher than diets B and D ($P < 0·05$).

On both these counts, therefore, peroxidation of the lipids of diets B and D during the exposure of less than 24 h in the trough would have been considerably slower during most of the experimental period than is shown in Fig. 1.

Table 2. *Composition of the skin lipids of the turkeys on diets containing different fats*

Diet	Lipid content of the skin (%)	Tocopherol content of the lipid (μg/g, as α-)
A (2·5% beef fat, control)	18·4	22
B (2·5% anchovy oil)	10·2	20
C (as B, +0·02% EMQ)	9·8	38
D (5·0% anchovy oil)	16·8	19

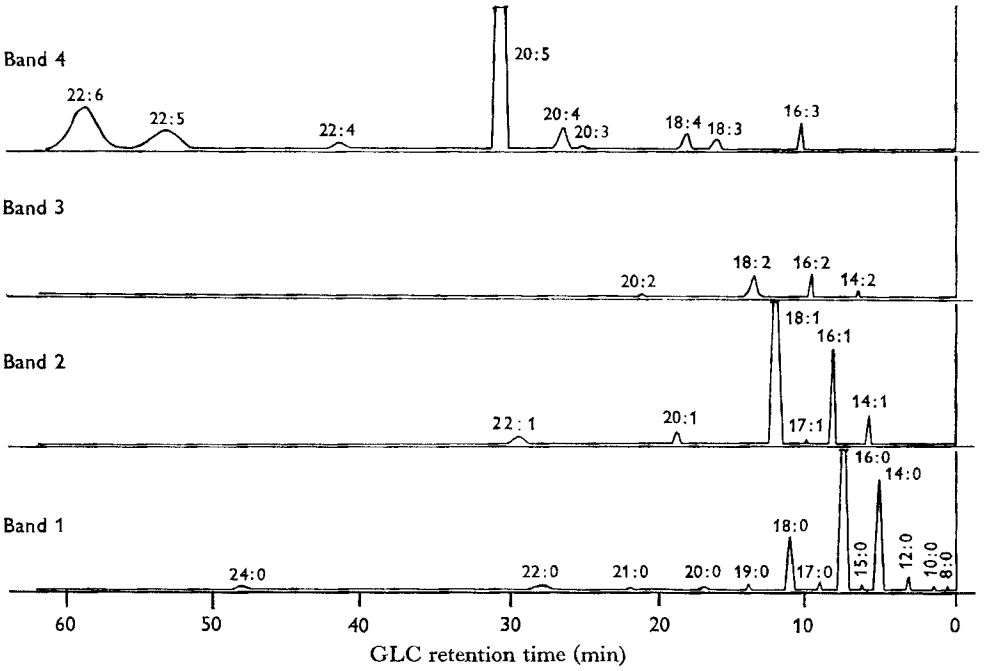

Fig. 2. Fractionation of anchovy oil methyl esters according to unsaturation on a silver nitrate–silica gel thin layer plate at 1°.

Performance of the birds. Growth and apparent health were normal on all four diets (Table 1). There did, however, appear to be a slightly inferior food conversion ratio ($P < 0.05$) and perhaps a slightly higher liver weight ($P > 0.05$) when 5% anchovy oil was given, as compared with values on the other three diets. Replacement of beef fat by anchovy oil slightly depressed storage of vitamin A in the liver, as previously observed (Lea *et al.* 1966), but diet D (5% anchovy oil), which also contained extra vitamin E, was not appreciably worse in this respect than diet B (2·5% anchovy oil). Vitamin E is known to conserve or protect vitamin A. Addition of EMQ with 2·5%

anchovy oil (diet C) restored the liver vitamin A to the level on the control (2·5% beef fat) diet A.

Analysis of the lipids. The fat contents of the skins of all the birds were rather low (Table 2), but negligible values for lipid phosphorus showed that the extracted lipid was virtually all neutral fat. Whereas fish oil may have marginally reduced the tocopherol content of the depot fat (diets B and D), EMQ (diet C) apparently raised it considerably. With the method of tocopherol estimation used, a recovery of 96% of added α-tocopherol was obtained and no interference by carotenoid with the determination was experienced.

Table 3. *Results of analysis of the anchovy oil methyl esters after hydrogenation*

	Hydrogenated esters	
Ester of acid	Found	Calculated from determined composition of the unhydrogenated esters (Table 4)
12:0	0·1	Trace
14:0	8·0	8·0
15:0	0·5	0·4
16:0	28·0	27·6
17:0	1·1	1·2
18:0	21·2	21·2
19:0	0·5	Trace
20:0	27·8	27·8
21:0	0·5	Trace
22:0	12·1	12·2
24:0	0·2	Trace
	Total 100·0	Total 98·4

Thin layer chromatography of the methyl esters of anchovy oil separated them cleanly into four fractions (Fig. 2), the fastest moving band (no. 1) containing the saturated components, followed by bands 2 and 3 of monoenes and dienes respectively, leaving polyenes in a fourth band near to the origin. To obtain sharp separation of dienes from polyenes it was essential to avoid overloading the plate.

Hydrogenation of the methyl esters of anchovy oil reduced their iodine value from 196·2 to 1·2 and greatly simplified the gas chromatographic pattern. Comparison of the analysis of the hydrogenated esters with that expected from the direct analysis of the unhydrogenated esters (using in the latter, identification facilitated by the separation achieved on the thin layer silver nitrate plate) gave satisfactory agreement (Table 3). Further confirmation of the validity of the identifications used was afforded by good agreement between the iodine value calculated for the fatty acid composition found (194·8) and that of the original ester mixture analysed (196·2).

The fatty acid compositions of the basal diet, of the fats added to it, and of the skin fats of the four groups of turkeys are summarized in Table 4. For ease of reference, the acids have been arranged in three groups according to whether they were major (> 1%), minor (1·0–0·1%) or very minor (< 0·1%) constituents.

Stability and flavour of the skin fats. The fats extracted from the skins of the birds

on diets B and D, despite precautions against autoxidation during handling and extraction, already showed small peroxide values (3·2 and 2·2 μmoles/g respectively), whereas those from the birds on diets A and C were zero. The colour of the extracted fats also increased progressively from A (white to cream) through C (definitely yellow) and B (deeper yellow) to D (very yellow). On incubation at 60° the fats developed peroxide in the same order (Fig. 3), the beef fat control diet (A) giving the most and the 5% anchovy oil diet (D) the least stable fat.

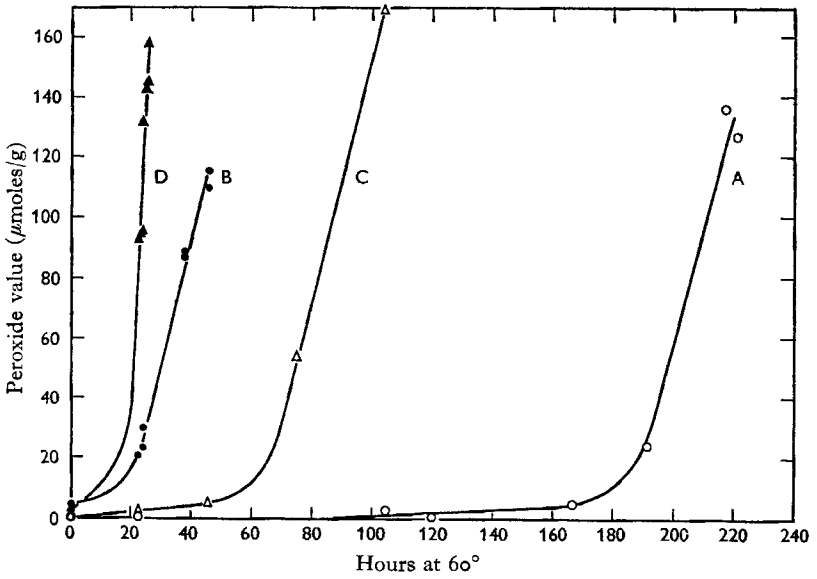

Fig. 3. Relative rates of autoxidation at 60° of the skin fats of turkeys on diets with different fats. Diet A, 2·5% beef fat; diet B, 2·5% anchovy oil; diet C, 2·5% anchovy oil+EMQ; diet D 5·0% anchovy oil.

Taste panel results on the roasted breast and leg meats from this experiment will be given in Part 2, together with the composition of the muscle lipids to which they directly relate. Full taste panel tests could not be done on the skin tissue alone, through lack of material, but it was obvious that fishy taints were present in all the groups given fish oil, with D certainly the most tainted and C probably less tainted than B.

DISCUSSION

Oxidation of the dietary lipids. Peroxidation of unstable lipids such as fish oils during the storage and feeding of experimental diets containing them can invalidate the results obtained unless a stabilizing antioxidant is also present, and this is not always practicable in view of possible nutritional effects. In the experiments of Rasheed, Oldfield, Kaufmes & Sinnhuber (1963), in which 15% fresh clay-bleached menhaden oil was given to rats in a semi-synthetic basal diet, toxic signs including anorexia, steatitis and death were attributed to rapid and extensive oxidation of the oil during 24 h exposure of the mixed diet before consumption. The same authors also

quote an experiment in which menhaden oil mixed in unspecified proportion with a synthetic diet increased in thiobarbituric acid (TBA) value from 32 to 1220 in 24 h at room temperature. On the other hand, we have previously found (Lea *et al.* 1966) that 1·5% anchovy oil incorporated into a normal type of cereal-based diet for feeding to turkeys did not oxidize appreciably in 24 h at 32°. The experiment now reported, in which 2·5% or 5·0% anchovy oil was included in a similar diet, represents an intermediate example, in that peroxidation of the oil during 24 h exposure at 27° was readily detectable (Fig. 1), but was too small to be nutritionally serious. Probable reasons for the diversity of these results are (*a*) the smaller proportions of oil used in our experiments, and (*b*) the protective action of natural antioxidants present in the cereal, soya and yeast constituents of the normal diet. Some of the nutritional effects of fish oils reported in the earlier literature may have been due rather to peroxidized fish oils.

Performance of the turkeys. The turkeys tolerated 5% of anchovy oil surprisingly well (Table 1), a result, however, in general agreement with the recent observation of Leong, Knobl, Snyder & Gruger (1964) that at 5% of the diet menhaden oil was equal to maize oil in feeding value for broiler chickens. Our result was, no doubt, influenced by the presence in the diet of 26 i.u./kg of added stabilized α-tocopheryl acetate and Leong *et al.*'s by the presence of 0·0166% EMQ.

Gas chromatographic analysis of the lipids. GLC provides a rapid means of separating and tentatively identifying the components of mixtures of fatty acid esters, but with so complex a mixture as a fish oil some supplementary aid is advisable to assist differentiation between close-running pairs such as the esters of 17:0 and 16:2, 18:4 and 20:1, and 20:5 and 22:1, where the opposing effects of increasing chain length and unsaturation can make identification difficult. For this purpose a preliminary fractionation of the ester mixture on a thin layer silica–silver nitrate plate, and an additional GLC analysis of the fully hydrogenated ester mixture were both found useful.

Quantitative operation can present more difficulty. Though a majority of users of the GLC technique have observed (or, more usually, assumed) proportionality between peak area and weight percentage (Horning, Ahrens, Lipsky, Mattson, Mead, Turner & Goldwater, 1964), some authors have found better agreement with mole percentage (Gruger, Nelson & Stansby, 1964), and the difference between these two methods of calculation can be large for a fish oil containing major components so far apart in molecular weight as C14 and C24. Still others (Kilgore & Luker, 1964; Pons & Frampton, 1965) have found it necessary to use a different conversion factor for each individual fatty ester. Because of these conflicting reports it has become fairly common practice to test the performance of the instrument and the conditions used on test mixtures of known composition, but these have often consisted of saturated esters only, or of saturated plus unsaturated esters up to linolenate (18:3). Only very rarely have esters of the 20:4, 20:5 and 22:6 acids been included, though these late-running components are the most likely to suffer loss under the conditions of high temperature or long time required to elute them. Gerson, McIntosh & Shorland (1964) reported losses of 51% and 86% during separation of the methyl esters of the C_{20} and C_{22} acids of shark-liver oil. In repeated runs with known mixtures con-

taining these highly unsaturated esters we found no evidence of any systematic loss or of deviation from proportionality between chart area and weight percentage, and no correction factors have been applied to any of the results reported in this paper.

Effect of dietary lipids on the composition of the skin fats. Replacement of 2·5% beef fat in the diet by the same amount of anchovy oil raised the iodine value of the skin

Table 4. *Fatty acid composition of the dietary fats, and of the skin fats of the four groups of turkeys receiving diets A–D with different fats*

Carbon number	Probable identity	Basal diet* IV 105	Beef fat† IV 47	Anchovy oil‡ IV 196	Skin fats A IV 68	B IV 99	C IV 94	D IV 133
			Major constituents (> 1%)					
14·00	14:0	1·1	3·5	7·8	1·7	4·5	4·3	5·9
16·00	16:0	22·4	24·0	14·0	25·0	26·3	27·0	22·4
16·49	16:1	1·8	4·7	9·2	7·0	6·7	6·6	7·9
17·20	16:2	—	—	2·1	—	0·7	0·7	1·1
17·70	16:3	—	—	2·0	—	0·7	0·6	1·1
16·95	17:0	Tr	1·4	1·2	0·7	1·0	1·0	1·0
18·00	18:0	6·7	18·9	2·2	10·3	8·7	8·0	6·1
18·40	18:1	19·3	41·0	17·3	37·4	20·8	21·4	19·7
18·95	18:2	43·2	2·9	1·2	13·9	15·8	15·5	13·9
19·80	18:3	4·7	0·4	0·4	1·3	1·5	1·7	1·6
20·20	18:4	—	—	3·0	—	0·8	0·7	1·3
20·35	20:1	Tr	Tr	2·1	0·5	1·2	1·2	1·5
21·75	20:4	—	—	2·2	—	0·5	0·5	0·7
22·48	20:5	—	—	23·4	—	5·9	4·9	10·0
24·55	22:5	—	—	3·0	—	2·2	2·2	2·6
24·85	22:6	—	—	7·3	—	1·9	1·7	3·0
			Minor constituents (1·0–0·1%)					
14·55	14:1	—	1·0	0·2	0·5	0·4	0·3	0·4
15·00	15:0	—	0·7	0·4	0·3	0·5	0·4	0·5
17·35	17:1, 18 br	—	0·8	—	0·4	—	—	—
20·00	20:0	Tr	Tr	Tr	0·5	0·4	0·6	0·6
22·20	22:1	—	—	0·1	—	0·1	0·1	0·1
23·55	22:4	—	—	1·0	—	Tr	Tr	Tr
			Very minor constituents (< 0·1%)					
10·00	10:0	—	Tr	Tr	Tr	Tr	Tr	Tr
12·00	12:0	—	Tr	Tr	Tr	Tr	Tr	Tr
14·75	15 br	—	Tr	NS	NS	NS	NS	NS
15·60	14:2, 16 br	—	0·4	Tr	0·1	Tr	Tr	Tr
16·80	17 br	—	0·8	NS	Tr	NS	NS	NS
19·02	19:0	—	Tr	Tr	Tr	Tr	Tr	Tr
19·50	20 br	—	Tr	Tr	Tr	Tr	Tr	Tr
19·65	19:1	—	—	Tr	Tr	Tr	Tr	Tr
20·90	20:2	—	—	Tr	—	Tr	Tr	Tr
20·95	21:0	—	—	Tr	Tr	Tr	Tr	Tr
21·30	20:3	—	—	Tr	—	Tr	Tr	Tr
22·00	22:0	—	—	Tr	Tr	Tr	Tr	Tr
24·00	24:0	—	—	Tr	—	Tr	Tr	Tr

Iodine values (IV) are the means of observed and calculated, rounded to nearest unit. Tr = < 0·1%; NS (no significant amount) = ≪ 0·1%; — = absent.

* Contributes 2·3% lipid to all diets.

† Contributes 2·5% to diet A. Also contains traces of 14 br and 19 br acids.

‡ Contributes 2·5% to diets B and C, 5·0% to diet D.

fat by about 30 units, and doubling the amount of anchovy oil raised it by another 30 units (Table 4).

The lipid of the basal diet, 2·3% in all four diets, was very much richer in linoleate than either the beef fat or the anchovy oil, and was presumably the main source of this acid in the skin fats; its concentration, therefore, did not vary very much. The beef fat contained more branched chain saturated acids, all in small amount, than did the other dietary fats; some of these branched chain acids were detectable also in the skin fat of the birds from group A. The anchovy oil contained seven major and two minor acids which were not present in either of the other dietary fats, and all of these were also present in the skin fats of groups B, C and D, which had received fish oil, but were absent from the skin fats of group A, which had not. One major acid (20:1), present at 2·1% in the anchovy oil and at the trace level only in the other dietary fats, was also present at much higher levels in the skin fats of the groups given fish oil.

Table 5. *Transference of individual fatty acids from dietary fish oil to the skin lipid of turkeys receiving diets B, C and D*

Acid	Concentration in anchovy oil (%)	Concentration in skin fat: No. of times level in diet B	C	D	As % of level in dietary lipid* B	C	D
16:2	2·1	13·3	13·3	10·5	64	64	76
16:3	2·0	14·0	12·0	11·0	67	58	80
18:4	3·0	10·7	9·3	8·7	51	45	63
20:1†	2·1	13·3	13·3	9·5	64	64	69
20:4	2·2	9·1	9·1	6·4	44	44	46
20:5	23·4	10·1	8·4	8·5	48	40	62
22:5	3·0	19·4	29·4	17·3	141	141	126
22:6	7·3	10·4	9·3	8·2	50	45	60
Mean (excluding 20:1)		11·6	10·7	9·0	55	51	65

* Total dietary lipid = 4·8% for diets B and C, 7·3% for diet D.
† Present also in skin fat of group A and calculations therefore based on increases.

The efficiencies with which the eight major fatty acids of fish oil were transferred from the dietary to the skin lipid followed a generally similar pattern, except for acid 22:5. When the fish oil level was 2·5% (diets B and C) the other seven acids were deposited in the skin fats at a level about eleven times greater than their concentrations in the whole diet, or approximately half their concentrations in the dietary lipid. When the fish oil content of the diet was raised to 5·0% (diet D) the corresponding figures were approximately nine times and two-thirds (Table 5). Acid 22:5, however, accumulated between two and three times as efficiently with diets B and C and nearly twice as efficiently with diet D. This could be the result of a more efficient deposition or less efficient mobilization of this particular acid, but it could, alternatively, be due to chain lengthening of a small proportion of the 20:5 acid, of which the anchovy oil contained as much as 23·4%, before deposition.

Marion & Woodroof (1963) gave 1% of menhaden oil to chickens and found approximately 1% of the 20:5 acid in the skin fat, but no acids of longer chain length were

detected, though they were present in the lipids of the muscle. Reed (1964) found that deposition of PUFA in the depot fat of rats did not follow the proportions in which they were present in the cod-liver oil given; though the 20:5 and 22:6 acids were present in the cod-liver oil in a ratio of nearly 2:1 the depot fat of rats receiving the oil showed elevated contents of hexaene but not of pentaene acids. The method (alkali isomerization) used for analysis of the depot fats gave fatty acid composition according to degree of unsaturation, but not according to chain length. In our experiment with turkeys there was no difference between the behaviour of the 20:5 and 22:6 acids.

Effect of diet on fat stability and flavour. The reason for the greatly reduced stability of the skin fats of the birds given fish oil as compared with the control group given beef fat (Fig. 3) can be seen from their compositions. The fats of groups A, B and D all contained approximately the same amount of tocopherol (Table 2) and the large differences in their stability therefore derived, presumably, from the higher levels of unsaturation in the groups given fish oil. The total concentrations of fatty acids with three or more double bonds in groups B, C and D were respectively 13·5, 12·3, and 20·3%, mainly pentaene and hexaene, whereas in group A the polyene level was only 1·3, all linolenate (18:3). The considerably greater stability of skin fat C as compared with B is not likely to have been due to its only slightly lower PUFA content, but was more probably due to a sparing of tocopherol by the antioxidant (EMQ) in this diet, which resulted in a tocopherol level nearly twice as high as in the other skin fats. Whether traces of EMQ itself might have been deposited in the skin fat of group C could not be determined with the methods of estimation and limited amounts of material available. The extra tocopherol in diet D did not result in increased deposition in the depot fat, presumably owing to a destructive effect of the high level of fish oil present in this diet.

Even dietary linolenic acid in sufficient quantity can cause 'off' flavours described as 'fishy' in roast turkey, as shown by Klose, Mecchi, Hanson & Lineweaver (1951) who gave 5% of linseed oil, but fishy flavours resulting from dietary additions of marine animal oils probably arise mainly from oxidative degradation of fatty acids still more highly unsaturated. The skin, because of its high fat content and maximum exposure to high temperature and oxygen during cooking, is particularly susceptible to the development of 'off' flavours of this kind.

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REFERENCES

Bowyer, D. E., Leat, W. M. F., Howard, A. N. & Gresham, G. A. (1963). *Biochim. biophys. Acta* **70**, 423.
Calvert, C. C., Desai, I. D. & Scott, M. L. (1964). *J. Nutr.* **83**, 307.
Carpenter, K. J., Lea, C. H. & Parr, L. J. (1963). *Br. J. Nutr.* **17**, 151.
Carpenter, K. J., Morgan, C. B., Lea, C. H. & Parr, L. J. (1962). *Br. J. Nutr.* **16**, 451.
Dam, H. & Søndergaard, E. (1964). *Z. ErnährWiss.* **5**, 73.
Desai, I. D., Calvert, C. C. & Scott, M. L. (1964). *Archs Biochem. Biophys.* **108**, 60.
Erickson, D. R. & Dunkley, W. L. (1964). *Analyt. Chem.* **36**, 1055.
Fry, J. L., van Walleghem, P., Waldroup, P. W. & Harms, R. H. (1965). *Poult. Sci.* **44**, 1016.
Gerson, T., McIntosh, J. E. A. & Shorland, F. B. (1964). *Biochem. J.* **91**, 11c.
Gruger, E. H. Jr, Nelson, R. W. & Stansby, M. E. (1964). *J. Am. Oil Chem. Soc.* **41**, 662.
Horning, E. C., Ahrens, E. H. Jr, Lipsky, S. R., Mattson, F. H., Mead, J. F., Turner, D. A. & Goldwater, W. H. (1964). *J. Lipid Res.* **5**, 20.
Kenney, H. E., Komanowsky, D. & Wrigley, A. N. (1965). *J. Am. Oil Chem. Soc.* **42**, 19.
Kilgore, L. T. & Luker, W. D. (1964). *J. Am. Oil Chem. Soc.* **41**, 496.
Klose, A. A., Mecchi, E. P., Hanson, H. L. & Lineweaver, H. (1951). *J. Am. Oil Chem. Soc.* **28**, 162.
Lea, C. H. (1952). *J. Sci. Fd Agric.* **3**, 586.
Lea, C. H. (1960). *J. Sci. Fd Agric.* **11**, 143.
Lea, C. H., Parr, L. J. & Carpenter, K. J. (1960). *Br. J. Nutr.* **14**, 91.
Lea, C. H., Parr, L. J., L'Estrange, J. L. & Carpenter, K. J. (1964). *Br. J. Nutr.* **18**, 369.
Lea, C. H., Parr, L. J., L'Estrange, J. L. & Carpenter, K. J. (1966). *Br. J. Nutr.* **20**, 123.
Leong, K. C., Knobl, G. M. Jr, Snyder, D. G. & Gruger, E. H. Jr (1964). *Poult. Sci.* **43**, 1235.
Marion, J. E. & Woodroof, J. G. (1963). *Poult Sci.* **42**, 1202.
Miller, D., Leong, K. C., Knobl, G. M. Jr & Gruger, E. H. Jr (1965). *Poult. Sci.* **44**, 1072.
Neudoerffer, T. S. & Lea, C. H. (1966). *J. Chromat.* **21**, 138.
Pons, W. A. Jr & Frampton, V. L. (1965). *J. Am. Oil Chem. Soc.* **42**, 786.
Rasheed, A. A., Oldfield, J. E., Kaufmes, J. & Sinnhuber, R. O. (1963). *J. Nutr.* **79**, 323.
Reed, S. A. (1964). *J. Sci. Fd Agric.* **15**, 399.
Wills, E. D. (1966). *Biochem. J.* (In the Press.)

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