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Nutritional effects of autoxidized fats in animal diets

4*. Performance of young pigs on diets containing meat meals of high peroxide value

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1. In two experiments, 100 pigs were fed from age 4 or 5 weeks to age 16–19 weeks on practical-type diets containing 10% of meat meal (15–17% lipid) of either low (3–17) or high (114–150 μmoles/g lipid) peroxide value. The cereal basis of the diet in the first experiment was wheat and maize and in the second barley. Other variants were the inclusion of a stabilized vitamin E supplement in the diet of one of the groups in Expt 1, the presence of 100 (Expt 1) or 250 (Expt 2) ppm of copper, and storage of a diet containing oxidized meat meal for 6–21 weeks before feeding to one of the groups in Expt 2.

2. Apart from an unusually high incidence of stomach ulcers which, however, were almost equally distributed between the control and oxidized fat groups and did not appear to affect the health and nutrition of the animals, the pigs grew well, with no significant difference between the groups in weight gain, food conversion ratio, liver weight, vitamin A storage in the liver, and aspartate aminotransferase and alanine aminotransferase activities in the serum.

3. Changes in the lipids of the diets during storage were studied and samples of back fat from the carcasses examined for fatty acid composition, tocopherol content and susceptibility to autoxidation. Moisture content appeared to be a major factor influencing the oxidative stability of the meat meals used. Tocopherol content and oxidative stability of the pig fat were highest in the group given a vitamin E supplement, and generally lower in Expt 2 than in Expt 1.

In previous papers of this series we have reported our failure to observe any serious effects of feeding 5% of oxidized beef fat in the diet to rats (Lea, Parr, L'Estrange & Carpenter, 1964) and chicks (L'Estrange, Carpenter, Lea & Parr, 1966) and of 1·5% oxidized fish oil to turkeys (Lea, Parr, L'Estrange & Carpenter, 1966) by comparison with control groups receiving fresh fat. In these experiments well-balanced practical-type rations were used, including supplements of vitamins A, D and E in stabilized form. The only consistent ill effect of the oxidized fat appeared to be a significant reduction of vitamin A storage in the liver, but in no instance was this sufficient to induce deficiency.

In the present paper the work has been extended to young pigs, with meat meals as source of the oxidized fat. There have been reports that the pig is susceptible to toxicity caused by oxidized fat, though the evidence is often rather obscure (cf. review by Grant, 1966). There have been reports from Sweden associating the clinical conditions, skeletal muscular dystrophy, liver necrosis and dietetic microangiopathy (MAP or mulberry heart disease) with the feeding of grain of poor oxidative stability,

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or grain in which oxidation has occurred following heating (Swahn & Thafvelin, 1962; Grant, 1961). Some or all of these conditions can usually be induced by high dietary levels of unsaturated fat such as cod-liver oil, and prevented by a dietary supplement of vitamin E or selenium (Grant, 1961; Lannek, Lindberg, Nilsson, Nordström & Orstadius, 1961). However, the tocopherol content of toxic grain was found not to be significantly lower than that of normal grain (Lannek, Lindberg, Nilsson, Nordström, Orstadius & Åberg, 1960), and Grant (1966) suggests that the condition MAP is induced especially by cereal with an easily disrupted anti-autoxidation system.

In this country under practical conditions the incidence of these disorders in pigs appears to be low, though there has been a report in which outbreaks of mulberry heart disease were associated with diets containing rancid fat (Oakley, 1963). It is possible that the low incidence in this country is due to the grains used having a more stable antioxidant system, and our own observation has been that even the addition of oxidized fat to cereal-based diets has usually failed to promote oxidation of the cereal lipid (Lea *et al.* 1964; L'Estrange *et al.* 1966). The danger of such oxidation, particularly if it destroys α-tocopherol in the process, could be expected to be more serious in pig than in poultry feeding, because it is not normal practice to supplement pig rations with stabilized vitamin E. We have studied changes in the fat fractions of our diets during storage since there is little information of this kind reported in the literature.

Meat meal was chosen as source of oxidized fat for the work reported here, partly because it is widely used in pig rations and partly because the lipid in meat meals used by the feeding industry often appears to be more or less oxidized. In both of the experiments described pigs were fed practical rations containing 10% meat meal, fresh or oxidized, from weaning for about 3 months. In each experiment the meat meal was of high lipid content (15–17%) and peroxide value (PV) of over 100 μmoles/g lipid. The nutritional effects of the oxidized fat have been small.

EXPERIMENTAL

Experiment 1

Fifty-four Large White piglets were weaned at 5 weeks of age and divided into three sets according to litter, sex and weight. Each set was then divided into three similar groups of six pigs each and the experimental diets A, B and C were allotted at random to one group in each set. Each diet, given *ad lib.* in dry meal form, was a practical-type ration, consisting of 90% of a common basal mix with 10% meat meal.

Basal diet. The 90 parts of basal diet were made up of ground wheat 36·25, maize meal 40, extracted soya-bean meal 11, casein 1·75 and a mineral–vitamin–antibiotic premix 1. The premix contributed to the final diet: vitamin A (as Rovimix A 50; Roche Products Ltd, Welwyn Garden City, containing 50000 i.u. vitamin A/g in a gelatin-based powder) 1000 i.u./kg; vitamin D_3 (as Rovimix D_3-100; Roche Products Ltd, containing 100000 i.u. vitamin D_3/g) 200 i.u./kg; riboflavine 2 ppm; iron (as $FeSO_4.7H_2O$) 100 ppm; copper (as $CuSO_4.5H_2O$) 100 ppm; zinc (as $ZnCO_3$) 50 ppm; NaCl 0·2% and oxytetracycline (as Terramycin; Pfizer Ltd, Sandwich, Kent) 8·9 ppm.

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The basal diet in stage 1 of the experiment (see below) contained 2·6% of chloroform–methanol extractable lipid of PV 2 μmoles/g; the corresponding values for stage 2 were 2·7% and 1 μmole/g.

Meat meals. The material used in diets B and C had been prepared from pork-processing waste by cooking and pressing, without solvent extraction, and was a batch that had already been rejected by a commercial analyst as 'unfit for feeding' because of the very high PV (105 μmoles/g) of its lipid. Analysis showed 49% crude protein, 17% lipid, 28% ash and 5·7% moisture. Diet C differed from B only in containing a supplement of vitamin E (Rovimix E; Roche Products Ltd, 10% α-tocopheryl acetate) 15 i.u./kg.

For control, we were unable to obtain a meal of sufficiently low PV; a freshly manufactured sample procured for the purpose had a PV of 37 μmoles/g lipid. A 'control' meal was therefore prepared by adding fresh lard and steamed bone flour to a fresh, commercially manufactured solvent-extracted meat meal (containing 6% lipid of PV 10 μmoles/g) to give, for use in diet A, a meal containing 15% lipid of PV 3 μmoles/g and the same protein and ash content as the meals used in diets B and C.

By the time a second batch of diet was required for extension of the experiment (see below) the solvent-extracted meal (which had been stored at 10–15°) had increased in PV to 31, giving a value, after admixture with fresh lard and bone flour, of 9 μmoles/g lipid (17% of the meal).

Feeding. The first batch of diet was fed from age 5 to 12 weeks, after which, no clear-cut result having emerged, a second batch of diets was prepared and feeding continued to slaughter at 16–18 weeks at a mean weight of 127 lb.

Experiment 2

Pigs. Fifty-four Landrace pigs were divided into nine groups, as in Expt 1, at 4 weeks and given one of two variants of an oxidized fat diet (E or F), or a control 'fresh fat' diet (D) for 12–15 weeks until slaughtered at mean weight of 128 lb.

Basal diet. The basal diet used was similar to that in Expt 1, except that (1) barley meal was given as sole cereal, in place of a mixture of wheat and maize meals, (2) copper was added at 250 ppm instead of at 100 ppm, and (3) supplementary vitamin E was not given in any of the treatments. The basal diet in this experiment contained 2·2% lipid, the PV of which ranged between 4 and 11 μmoles/g during storage.

Meat meals and conditions of feeding. A freshly made meat meal, containing 50% crude protein, 29% ash and 16% lipid of PV 16 μmoles/g, was held under conditions favouring oxidation (p. 381) until it reached a PV of 114 μmoles/g. Part of this oxidized meal was then mixed with nine parts of basal diet and the whole stored at 10–20° for 6 weeks before feeding (and during feeding) to the pigs of group F.

The pigs of group E received diet mixed weekly from the basal diet (stored at 10–20°) and the oxidized meat meal (stored at −10°). During storage at −10° for 6 weeks before use the PV of the oxidized meal increased from 114 to 133, and during the next 15 weeks, to the end of the feeding period, it rose further to 150 μ moles/g lipid.

The 'control' pigs (group D) received a meal prepared from solvent-extracted

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meat meal, bone flour and fresh lard, to have the same proximate analysis as the oxidized meal; though stored at $-10°$ its PV rose from 3 to 17 μmoles/g lipid during the feeding period.

Methods of analysis

Determinations of lipid content (chloroform-soluble portion of chloroform–methanol extract), free fatty acids, peroxide, carbonyl (both direct and after iodometric reduction of peroxides) and iodine values (both of the lipids as extracted and of the total fatty acids prepared from them) were as described in previous papers in this series. Stability (Lea *et al.* 1964) and tocopherol (Neudoerffer & Lea, 1966) determinations were made on fat extracted with diethyl ether from back adipose tissue adjacent to and removed with the tail, and these fats were also analysed for fatty acid composition by gas liquid chromatography (Neudoerffer & Lea, 1966).

Performance of the pigs and examination of the carcasses

Weight increase and food consumption were recorded at weekly intervals throughout the experiment. Blood samples were taken occasionally from individual pigs, from the ear vein in Expt 1 and from the vena cava in Expt 2, and also from all pigs at slaughter. Levels of serum aspartate aminotransferase (Asp. T) and of serum alanine aminotransferase (Al. T) were determined in individual samples as previously described (Lea *et al.* 1966). The resistance to haemolysis of the erythrocytes was measured by the dialuric acid test (Bunyan, Green, Edwin & Diplock, 1960) in forty-two pigs in Expt 2 during the feeding trial. At slaughter, the liver was examined for necrotic lesions, the stomach for ulceration and the heart for macroscopic signs of MAP. Two pigs from each treatment in each experiment were examined for signs of muscular dystrophy in the hind-leg muscle. The livers were weighed and vitamin A was determined in each liver as previously described (Lea *et al.* 1964).

RESULTS

Stability of the lipid in meat meal

As already stated, several of the samples of meat meal procured for the feeding experiments showed oxidative instability, even at $-10°$. On the other hand, a commerical meal (moisture content 6·6%), which had the comparatively high PV of 60 μmoles/g lipid when received, showed little change in this value on further storage for 6 weeks at 10–15° (Fig. 1, A1); this was true also for the oxidized meal of PV 105 μmoles/g lipid fed in Expt 1 (Fig. 1, B1).

A freshly manufactured meal, of moisture content 4·8%, had a PV of 16 μmoles/g lipid when received, and a sample of this meal oxidized steadily when stored in a polythene bag with ample headspace, to reach a PV of 145 μmoles/g lipid after 6 weeks (Fig. 1, A2). But when, 2 weeks later, an attempt was made to oxidize the main bulk of this meal by exposing it to the atmosphere, on a concrete floor with raking at intervals, the rise in PV soon came to a stop (Fig. 1, A3); much of the small initial rise in PV observed probably occurred before the meal was spread on the floor.

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It seems likely that cessation of oxidation was associated with absorption of moisture, the content of which had risen to 5·9% after a total storage time of 6 weeks.

The oxidized meal fed in Expt 2 was therefore prepared by storing fresh meal (initial PV 16 µmoles/g lipid) in large polythene sacks partly filled, with shaking at intervals. Under these conditions the rise in PV was quite rapid (Fig. 1, B2). The meal in about half of the sacks was appreciably darker in colour than the remainder, and

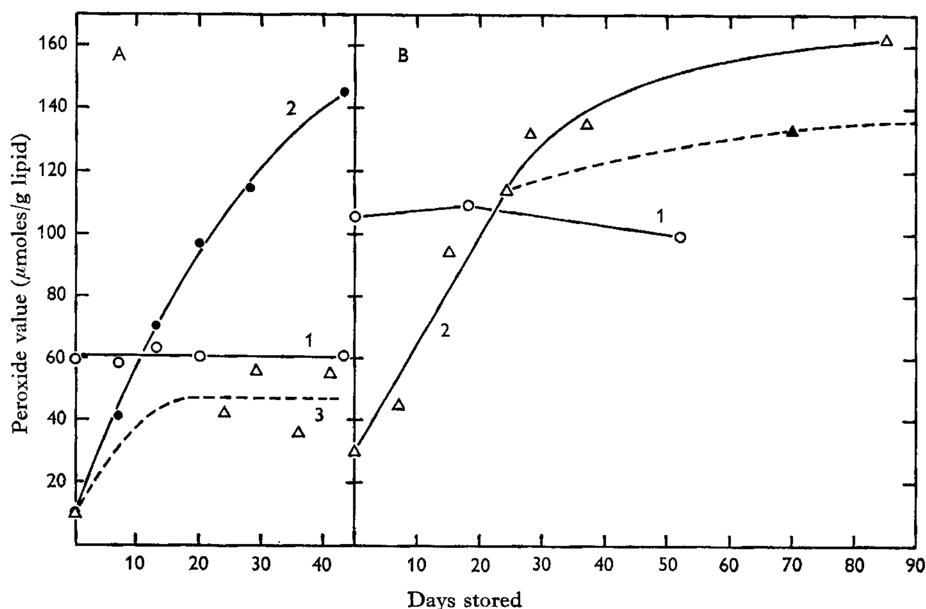

Fig. 1. Oxidation of lipid in stored meat meals. (A) (1) Commercial sample (14% lipid, 6·6% moisture) stored in sacks at 10–15°, ○——○. (2) Fresh commercial sample (14% lipid, 4·8% moisture) stored in polythene bags at 15° with headspace and occasional shaking, ●——●. (3) Same sample as 2, but stored 24 days in full sacks and thereafter in 6 in. layer on floor exposed to atmosphere, with moisture content rising to 5·9%, △ - - - △. (B) (1) Oxidized commercial sample (17% lipid) condemned by analyst, fed in Expt 1; stored in sacks at 10–15°, ○——○. (2) Fresh commercial meal (16% lipid, 4·4% moisture) stored at 15° in polythene bags with headspace and occasional shaking, △——△. After 24 days most of this meal was repacked for feeding in Expt 2, in full polythene bags and stored at −10°, ▲ - - - ▲.

this darker meal had a consistently lower PV than the lighter: values after 0, 18 and 52 days were respectively 18, 38 and 81 for the darker and 41, 52 and 107 µmoles/g lipid for the lighter meal. The means of these values have been plotted in Fig. 1, B2. The moisture contents of the two fractions were 4·3 and 4·5% respectively; the lower moisture, darker colour and lower PV of the former may have been due to stronger heating during manufacture. At 24 days the two fractions were thoroughly blended together in a power mixer (PV now 114 µmoles/g lipid) and repacked in polythene bags for further storage, a small part at 15°, the greater part at −10° for use in Expt 2. The PV of the portion at 15° continued to increase though at a decreasing rate, to reach a value of 162 µmoles/g lipid after a total storage time of 12 weeks (Fig. 1, B2); its moisture content was now 4·7%. Despite the low storage temperature the material

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at $-10°$ continued to oxidize slowly: at the commencement of feeding it had reached a PV of 133 and by the end of the experiment (i.e. after 21 weeks at $-10°$) the PV was 150 μmoles/g lipid.

Changes in the diets during storage

Expt 1. The PV of the lipid of the control diet (A) remained low (3–5 μmoles/g lipid) throughout the experiment (Table 1). The commercial sample of oxidized meat meal fed in diets B and C had a PV of 105 μmoles/g lipid when received, and this value remained approximately constant during storage of the meal in the piggery at 0–15°, while feeding was in progress (Fig. 1, B1; Table 1). Direct carbonyl determinations on the meal showed only a slight rise, from 120 initially to 143 μmoles/g lipid after 7 weeks (Table 1).

The two batches of basal diet used in the first and second stages of feeding had approximately the same lipid content (2·6 and 2·7% and PV 2 and 1 μmoles/g lipid). Mixing the oxidized meat meal with the basal diet gave calculated 'initial' PVs of 45 (stage 1) and 41 (stage 2) μmoles/g lipid in the mixed diets, the lipid contents of which were about 4% (Table 1). During storage of the mixed diets in the piggery at 0–15° the PV decreased to 32 after $2\frac{1}{2}$ weeks and to 22 after 7 weeks (stage 1), and to 21 after 5 weeks (stage 2). Direct carbonyl values tended to rise slightly during storage of the diets; changes in carbonyl value after reduction of peroxide were small and erratic. Observed changes in iodine value were small, indicating the absence of any major oxidative destruction of unsaturated fatty acids such as is found, for example, in stored fatty fish meal (Lea, Parr & Carpenter, 1958). There was some increase in free fatty acid content of the lipid in all three diets.

Expt 2. The PV of the lipid of the control diet (D) was slightly higher (7–11 μmoles/g) in this experiment, partly owing to a higher value for the lipids of the barley-based basal diet and partly owing to oxidation of the 'fresh' meat meal during storage at $-10°$, during which the PV of its lipid rose from 3 to 17 μmoles/g (Table 2).

The PV of the oxidized meal in diet E ranged from 133 at the beginning to 150 at the end of feeding, corresponding to calculated PVs of 66 and 70 in the total lipid of the diet. Neither the control nor the oxidized fat diet changed appreciably during the week it was stored at the piggery after mixing, as shown by the values given in Table 2 for a specimen week, about the middle of the feeding period.

Mixing oxidized meat meal, of PV 114 μmoles/g lipid, with basal diet, to produce diet F, gave a calculated initial PV of 53 μmoles/g total lipid of the diet, but this had fallen to 28 by the time feeding commenced 6 weeks later and to 21 by the end of the feeding period (Table 2). Despite this fall in PV there was an indication in the falling iodine values that some degree of further oxidation had probably occurred, both in the basal and in the mixed diet during storage at 10–20°, though its extent could not have been great.

Performance of the pigs

Expt 1. Of the fifty-four piglets which started the experiment at an average weight of 21·6 lb, four died and two were taken out of the experiment before it ended. On diet A two died from acute enteritis; on diet B one died from MAP and another was

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Table 1. *Expt 1. Changes in the lipids of the diets during the two stages of feeding (0–7 and 7–12 weeks)**

Diet	Analysis†	Initial composition (stage 1) Meat meal (10 %)	Initial composition (stage 1) Basal diet (90 %)	Initial composition (stage 1) Whole diet (calculated)	Whole diet stored 7 weeks	Initial composition (stage 2) Meat meal (10 %)	Initial composition (stage 2) Basal diet (90 %)	Initial composition (stage 2) Whole diet (calculated)	Whole diet stored $5\frac{1}{2}$ weeks
A (control)	Lipid content (%)	15	2·6	3·8	3·8	17	2·7	4·1	4·1
	PV (μmoles/g lipid)	3	2	3	5	9	1	4	5
	FFA (% in lipid)	3	19	13	26	4	10	8	13
	IV of lipid	55	120	95	96	55	121	94	93
	Carbonyl (μmoles/g lipid)	20	30	26	31	23	25	24	35
B and C‡ (oxidized fat)	Lipid content (%)	17	2·6	4·0	4·0	17	2·7	4·1	4·1
	PV (μmoles/g lipid)	105	2	45	22	99	1	41	21
	FFA (% in lipid)	5	19	13	22	5	10	8	12
	IV of lipid	54	120	92	92	51	121	92	91
	Carbonyl (μmoles/g lipid)	120	30	68	78	143	25	74	96

* The pigs were killed after 11–13 weeks.

† PV, peroxide value; FFA, free fatty acids; IV, iodine value. Owing to the limited accuracy of sampling and lipid extraction and, in some instances, of determination, many of the analytical values given in this paper have been rounded off to the nearest unit.

‡ Differences between diets B and C (without and with added α-tocopheryl acetate respectively) were small and mean values are reported.

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Table 2. *Expt 2. Changes in the lipids of the diets during storage before feeding and during the feeding period* (6–21 *weeks*)*

Diet	Analysis	Initial composition			Composition at 6 weeks			Composition at 21 weeks			Mixed diet‡	
		Meat meal (10 %)	Basal diet (90 %)	Whole diet (calculated)	Meat meal (10 %)	Basal diet (90 %)	Whole diet (calculated)†	Meat meal (10 %)	Basal diet (90 %)	Whole diet (calculated)†	Initial	After 1 week
D (control):	Lipid content (%)	—	2·2	—	16	2·2	3·6	16	2·2	3·6	—	—
mixed weekly from 'fresh'	PV (μmoles/g lipid)	—	4	—	3	11	7	17	6	11	10	8
meat meal stored at −10° and	FFA (% in lipid)	—	11	—	5	24	16	20	38	30	22	25
basal stored at 10–20°	IV of lipid	—	111	—	58	111	87	58	106	85	—	—
	IV of total fatty acids	—	126	—	—	—	98	—	—	—	—	—
	Carbonyl (μmoles/g lipid)	—	44	—	14	67	43	58	77	68	46	43
E (oxidized fat):	Lipid content (%)	16	2·2	—	16	2·2	3·6	16	2·2	3·6	—	—
mixed weekly from oxidized	PV (μmoles/g lipid)	114	4	—	133	11	66	150	6	70	66	69
meat meal stored at −10° and	FFA (% in lipid)	2	11	—	2	24	14	2	38	22	19	23
basal stored at 10–20°	IV of lipid	57	111	—	57	111	87	55	106	84	—	—
	IV of total fatty acids	—	126	—	—	—	97	—	—	92	—	—
	Carbonyl (μmoles/g lipid)	121	44	—	132	67	96	160	77	114	116	96
F (oxidized fat):	Lipid content (%)	16	2·2	3·6	—	—	3·6	—	—	3·6	—	—
oxidized meat meal mixed	PV (μmoles/g lipid)	114	4	53	—	—	28	—	—	21	—	—
with basal and stored at	FFA (% in lipid)	2	11	7	—	—	20	—	—	35	—	—
10–20° for 6 weeks before	IV of lipid	57	111	88	—	—	90	—	—	82	—	—
feeding and throughout	IV of total fatty acids	—	126	99	—	—	100	—	—	94	—	—
feeding	Carbonyl (μmoles/g lipid)	121	44	78	—	—	84	—	—	153	—	—

* PV, peroxide value; FFA, free fatty acids; IV, iodine value. The pigs were killed during the period 18–21 weeks.

† The values for diet F, except lipid content, are determined ones.

‡ These are determined values for batches of whole diet mixed near the middle of the feeding period and sampled immediately and then after 1 week's storage in the piggery at 10–20°.

removed because of prolapsed rectum; on diet C one died as a result of torsion of the intestine and another was removed because of prolapsed rectum. All these disorders, except for one, were upsets of the digestive tract and the losses were evenly distributed between the treatments.

The performance of the remaining animals, which all appeared healthy and grew well, is summarized in Table 3. There were no significant differences between treatments in weight gain or food conversion ratio after 11 weeks on the diets, though mean weight gain on diet B (oxidized meat meal without vitamin E supplement) was slightly lower than on the other two treatments. On subdividing the 11-week period into three parts, i.e. 0–3, 3–7 and 7–11 weeks, it is seen that practically all the difference occurred in the 3–7-week period when it reached significance ($P < 0.05$). There was considerable individual variation in the weight gains of the pigs on each treatment, probably owing to their immaturity when grouped.

Table 3. *Expt 1. Performance of the pigs. Mean values for eight castrated males and eight females per treatment, receiving diets containing* 10% *fresh meat meal, or* 10% *oxidized meat meal with or without a supplement of vitamin E*

	Diet A (fresh meat meal)	Diet B (oxidized meat meal)	Diet C (oxidized meat meal with vitamin E)	SE*
Response of the pigs from 5 to 16 weeks of age				
Live-wt gain (lb/pig day) in feeding period:				
0–3 weeks	0·62	0·64	0·61	±0·023
3–7 weeks	1·03	0·87	1·06	±0·032
7–11 weeks	1·67	1·66	1·72	±0·051
0–11 weeks	1·16	1·09	1·18	±0·027
Food conversion ratio 0–11 weeks	2·55	2·57	2·53	±0·022
Measurements at slaughter at 16–18 weeks of age				
Liver wt (as % body-wt)	1·96	1·98	1·91	±0·037
Vitamin A in liver (i.u. $\times 10^{-3}$/liver)	29·1	27·1	25·8	±2·3
Serum Asp. T† (International Units)	42·6	51·8	47·4	±3·9
Serum Al. T† (International Units)	26·4	26·5	26·6	±1·6
Stomach ulcers: incidence (no. of pigs)	7	10	9	±2
severity‡ (no. of stars)	17	23	32	—

* Standard errors of treatment means, calculated from pen means and based on four degrees of freedom. In an analysis of variance only the weight gains of the pigs from the 3rd to the 7th week of the feeding period were found to be significantly affected by treatment, with those for diet B lower than the others.

† Asp. T, aspartate aminotransferase; Al. T, alanine aminotransferase.

‡ The severity of each ulcer was rated by number of stars up to a maximum of five.

Levels of Asp. T and Al. T were not abnormally high in any of the blood samples taken during the feeding trial. At slaughter the level of both enzymes in each individual pig was measured and again no abnormally high value was found, nor was any significant treatment effect observed. There was no significant difference between treatments in liver weight (as % of body-weight) or in vitamin A reserves in the liver.

A number of the pigs were found to have stomach ulcers at slaughter: all of these occurred in the fundal mucosal ridges as linear shallow black areas of necrosis, usually accompanied by reddening of the mucous membrane with increased out-

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pouring of mucus. The incidence of the ulcers appeared to be fairly evenly distributed between the treatments, though with a tendency for them to be fewer and less severe on control diet A (Table 3).

A large proportion of the livers showed the 'milk spots' associated with the presence of *Ascaris lumbricoides* but there was no hepatic necrosis. There were no gross changes in any of the kidneys examined.

Expt 2. All but one of the fifty-four piglets which started in this experiment at an average weight of 16 lb survived until slaughter, after 12–15 weeks on the diets. The one which died was on diet F; it failed to gain weight and died after 7 weeks, from pneumonia. The remaining animals all appeared healthy, though one, on diet E, became lame in one hind-quarter towards the end of the experiment. The performance of the pigs is summarized in Table 4.

Table 4. *Expt* 2. *Details of treatments and mean performance of the pigs in each group.* (*Three pens of six pigs/treatment*)

	Diet D	Diet E	Diet F	SE*
Meat meal:				
Period of oxidation at 15° (weeks)	0	3·5	3·5	—
Peroxide value (μmoles/g lipid) when mixed into the diet	3–17	133–150	114	—
Whole diet:				
Time of storage at 10–20° between mixing with meat meal and feeding (weeks)	0–1	0–1	6–21	—
Peroxide value of total lipid in the mixed diet at the time of feeding (μmoles/g)	7–11	66–70	28–21	—
Response of the pigs from 4 to 16 weeks of age:				
Live-wt gain (lb/pig day)	1·16	1·10	1·09	±0·051
Food conversion ratio	2·35	2·46	2·42	±0·050
Measurements at slaughter at 16–19 weeks of age:				
Liver wt (as % body-wt)	1·70	1·71	1·75	±0·042
Vitamin A in liver (i.u. × 10^{-3}/liver)	18·5	16·0	20·9	±1·45
Serum Asp. T† (International Units)	45·9	35·7	41·5	±2·68
Serum Al. T† (International Units)	27·5	24·2	24·6	±1·85
Stomach ulcers: incidence (no. of pigs)	8	11	11	±2
severity‡ (no. of stars)	18	22	22	—

* Standard errors of treatment means, calculated from pen means and based on four degrees of freedom. In an analysis of variance there were no significant treatment effects.

† Asp. T, aspartate aminotransferase; Al. T, alanine aminotransferase.

‡ The severity of each ulcer was rated by number of stars up to a maximum of five.

There was no significant difference between treatment means after 12 weeks on the diets, either for weight gain or food conversion ratio, both of which were satisfactory. At slaughter, there was no significant difference in liver weight or in vitamin A reserves in the liver. Levels of serum Asp. T and Al. T were normal in all individual pigs at slaughter and there was no significant difference between treatment means: all blood samples taken during the experiment gave normal values for both enzymes. The erythrocyte haemolysis test was negative in all samples tested.

There was again a high incidence of stomach ulcers of the same type as those observed in Expt 1; they were fairly evenly distributed between treatments, though again there tended to be fewer in the control pigs of group D. The mean packed cell volume

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(PCV) in twelve pigs with ulcers was 43·3 ml/100 ml compared with 42·3 in six not showing ulcers, the corresponding values for haemoglobin were 13·5 and 13·3 g/100 ml, indicating that the ulcers were not severe enough to cause anaemia.

The sciatic nerve in the leg of the pig which was lame showed a small amount of demyelination and some neuronophagia, which suggested pantothenic acid deficiency. There was no evidence of muscular dystrophy, and the serum Asp. T and Al. T concentrations were normal.

Table 5. *Fatty acid composition (% by weight), tocopherol content and stability of the pig fats of the six dietary groups A–F*

	Expt 1			Expt 2		
Fatty acid	A	B	C	D	E	F
14:0	1·2	1·3	1·3	1·4	1·4	1·3
16:0	25·3	24·3	23·2	23·8	23·7	24·1
16:1	4·4	4·0	4·5	5·0	5·1	5·3
18:0	11·0	10·3	9·5	8·7	8·5	8·7
18:1	46·0	47·5	49·8	50·2	51·3	51·2
18:2	11·1	10·6	9·1	7·9	7·9	7·4
Total	99·0	98·0	97·0	97·0	97·9	98·0
Tocopherol (μg/g)	2·4	2·3	4·8	0·5	0·6	1·4
Stability (h at 60° to peroxide value 25 μmoles/g)	72	52	107	37	35	37

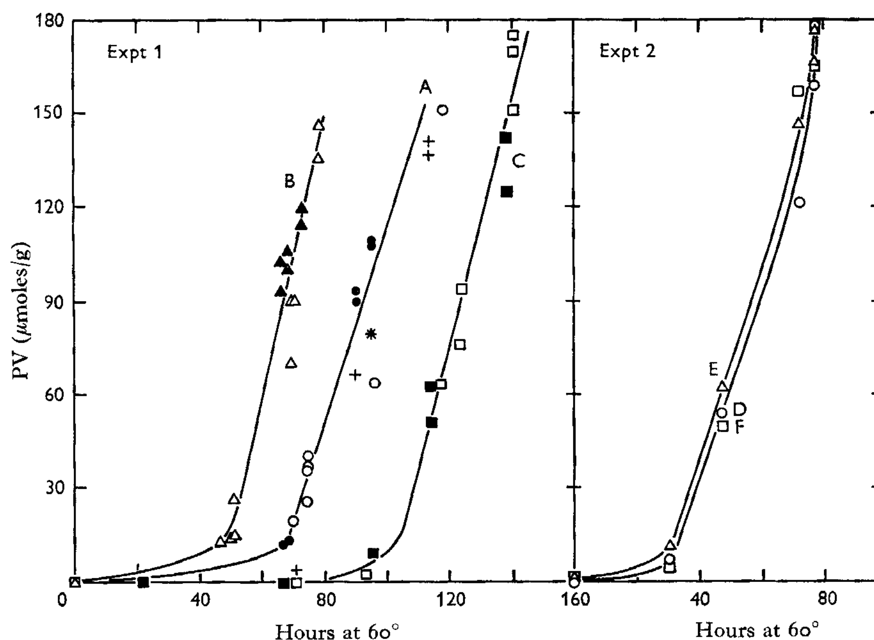

Fig. 2. Relative rates of autoxidation of the extracted pig fats at 60°. Replicate extracts are given seprrate symbols in Expt 1: diet A, ○ ● +; B, △ ▲; C, □ ■. In Expt 2: diet D, ○; E, △; F, □. Details of the diets are given on pp. 378, 379.

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Composition and stability of the pig fats

The proportions of the six major fatty acids, accounting for 97–99% of the total acids of the pig fats, are given in Table 5. Peaks attributable to at least eight other acids, present at very low concentrations and mostly of uncertain identity, have not been included. The tocopherol contents of the fats are also given in Table 5 and their stabilities towards autoxidation in Fig. 2 and Table 5.

The fact that slight differences in the proportion of stearic, oleic and linoleic acids were more noticeable between the experiments than between the treatments suggests that changing the basal diet from mainly wheat and maize to mainly barley (and increasing the copper content from 100 to 250 ppm) had more effect than feeding oxidized meat meal in place of fresh, the effect of this change on the fatty acid composition of the body fat being negligible.

The determination of tocopherol at the very low levels present in these pig fats was not very accurate, but the values suffice to show that the levels were generally lower in Expt 2 than in Expt 1, and that supplementation of the oxidized fat diet with stabilized tocopherol in Expt 1 raised the level in the pig fat (Table 5).

The oxidative stabilities of the pig fats were also low, especially in Expt 2. There was no difference attributable to the more oxidized dietary fat in Expt 2, and in Expt 1 the difference found was not great in relation to the limited accuracy of the determination (Fig. 2). The pigs that received the tocopherol supplement produced the most stable body fat.

DISCUSSION

Effects of the diets on the pigs

In neither of the two experiments were any clear-cut adverse effects observed that could be attributed to the oxidized meat meal. The piglets were purposely weaned on to the experimental diets at the early age of 4 or 5 weeks in order to accentuate any dietary stress, and losses in Expt 1 due to disorders of the digestive tract were high (11%), but they were evenly distributed between the treatments; losses in Expt 2 were only 2%. The surviving pigs accepted the rations readily, grew at normal rates and their food conversion ratios were quite good.

As in the investigation with turkeys (Lea *et al.* 1966), weight gains of the pigs were apparently slightly lower on the peroxidized lipid diet (without vitamin E supplement) than on the control diet but, as with the turkeys, the effect did not reach significance. In the previous experiments inclusion of 5% oxidized beef fat in the diet of rats (Lea *et al.* 1964) or chicks (L'Estrange *et al.* 1966) or of 1·5% oxidized fish oil in the diets of turkeys (Lea *et al.* 1966) had appreciably decreased storage of vitamin A in the livers, but no significant differences could be detected between the pigs on the oxidized meat meal and control groups; the proportion of oxidized pig fat in these rations (1·6–1·7%) was apparently not sufficient to destroy any major part of the vitamin A after release from its protective gelatin matrix in the intestine. Storage of vitamin A in the livers was appreciably less in Expt 2 than in Expt 1. It has already been pointed out (Carpenter, L'Estrange & Lea, 1966) that the lipid peroxide levels in diets should preferably

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be referred to the diet as a whole rather than to the fat itself; in the present experiments the dietary lipid peroxides at no time exceeded 2·5 μmoles/g total diet.

The levels of Asp. T and Al. T enzymes in the blood serum are known to increase to several times above the normal with the development of muscular or hepatic dystrophy (Orstadius, Wretlind, Lindberg, Nordström & Lannek, 1959), but no significant increase in the concentrations of these enzymes was found in the present experiments. Nevertheless, there was some evidence, both microscopic and macroscopic, of the presence of muscular dystrophy in a very mild form, not resulting in lameness, in the legs of three of the animals examined in Expt 2; one of them was on the fresh meat meal diet. The lameness in the one pig that developed it was of the type seen in pantothenic acid deficiency.

The very high incidence of a mild form of stomach ulcer was an unsatisfactory feature of both experiments, particularly as peptic or duodenal ulceration has been reported in man as a result of long-term ingestion of peroxidized fat (Horwitt, 1962), but the ulcers in the pigs occurred almost as frequently and severely in the controls as in the experimental groups. Moreover, the mean weight of all the pigs with ulcers was 129 lb compared with 125 lb for those without ulcers and this, together with the absence of any signs of anaemia in the affected animals, indicates that the condition had not seriously affected the pigs. These ulcers, in the fundal region of the stomach, seemed to be of a different type from those occurring in the oesophageal region of the stomach, which occur frequently in pigs in some parts of the USA (Muggenberg, Reese, Kowalczyk, Grummer & Hoekstra, 1962) and which have been observed to develop when very high levels of copper are fed (Allcroft, Burns & Lewis, 1961; Buntain, 1961).

Changes in the lipids of the diets

There was some evidence that the lipid of the barley-based basal diet in Expt 2 oxidized to some extent during storage, probably under the influence of its very high level of copper (250 ppm). The PV of the extractable lipid, which rose from 4 to a maximum at about 13 and then fell again to 6 μmoles/g, gave no clear information; the carbonyl value continued to rise (Table 2). Detailed fatty acid analyses of the lipids were not made during storage of the diets, but the observed fall in iodine value of 5 or 6 during storage and the lower linoleate content of the body fats produced by the pigs in Expt 2 suggest that some oxidative destruction of this acid probably occurred during storage of the basal diet.

High levels of oxidized and particularly of actively oxidizing fat are liable to destroy part of the available (natural) vitamins E and A, and the vitamin E nutrition of the pigs, particularly in Expt 2, was probably suboptimal as indicated by the low tocopherol contents and oxidative stabilities of their body fats, as well as by the cases of mild muscular dystrophy.

Meat meal is obviously a difficult material to protect from lipid autoxidation, and the beneficial effect of storage at $-10°$ was very limited. On the other hand, the observation that exposure to the atmosphere virtually arrested autoxidation in one meal, probably owing to the absorption of moisture, suggests that careful storage of

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the meal, in, for example, sacks of polythene or other 'moisture-proof' material, may be disadvantageous from the viewpoint of oxidative stability of the lipid, and that more careful control of the moisture content of the meal to a relatively high level by the manufacturer might well be useful. It has long been known that the lipids of many 'dry' products such as milk powder, wheat flour and fish meal tend to autoxidize more rapidly at low than at moderate moisture contents and the same is true of dehydrated meat (Lea, 1943), but the mechanisms and factors involved are still not fully understood.

Carbonyl determination after reduction of peroxides

As previously suggested (Lea *et al.* 1966), determination of carbonyl groups after reduction of interfering hydroperoxides should have advantages over determination of PV or of the direct carbonyl value as a measure of lipid oxidation, and many determinations by this technique were carried out in the present work, but with disappointing results. Speedy handling is essential, because of the rapidity with which the lipid reoxidized after reduction, and losses by emulsification were considerable. Though the weight of lipid recovered could readily be ascertained, selective losses of the more polar carbonyls apparently occurred and results were erratic. It may be possible to overcome these difficulties but, in its present form, the method is not satisfactory for routine use.

We are grateful to the staffs of the Department of Veterinary Medicine and of the Veterinary Investigation Centre, Cambridge, for post-mortem examinations of the animals that died and for the determination of packed cell volumes and haemoglobin concentrations in Expt 2. We are also grateful to Mrs Jennings, Department of Pathology, for examination of all the slaughtered animals; she will be reporting separately on the nature of the stomach ulcers seen in this work.

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