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Adverse effects of cyclopropenoid fatty acids

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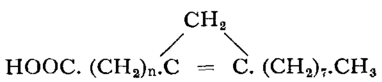
Sterculic (I: n=7) and malvalic (I: n=6) acids are the only naturally occurring cyclopropenoid fatty acids so far encountered, and sterculic appears to be the more abundant acid.



I

They occur in plant lipids mainly as glycerides, and the possible presence of cyclopropenoid material in some fifty plant species has been reported (Carter & Frampton, 1964; Phelps, Shenstone, Kemmerer & Evans, 1965). The species containing the greatest quantity of these acids are given in Table 1.

Table 1. *Vegetable oils which contain the greatest quantity of cyclopropenoid fatty acids (% of total fatty acids)*

Species	Sterculic acid	Malvalic acid	Reference
Sterculia macrophylla (seed)	63	4	F. D. Gunstone (1964), unpublished work
S. parviflora (seed)	45	19	F. D. Gunstone (1964), unpublished work
S. foetida (seed)	55	7	Wilson, Smith & Mikolajczak (1961)
S. foetida (seed)	Total 44–45·6		Earle, Melvin, Mason, Van Etten, Wolff & Jones (1959); Harris, Magne & Skau (1963)
Bombacopsis glabra (seed)	35	Nil (<0·5)	Cornelius, Hammonds & Shone (1965)

Quantitative analysis for individual cyclopropene acids is rather complicated and there is no one simple method which can be adopted, but quantitative estimation of total cyclopropenoid content can be achieved most conveniently by titration (Durbetaki, 1956; Harris, Magne & Skau, 1964), to a level of 0·01%. Qualitative detection has been possible for some time by the Halphen test (Halphen, 1897; Deutschman & Klaus, 1960), which appears to be specific for cyclopropenoid material with an unsubstituted ring methylene group (Nordby, 1963) and involves the use of 1% sulphur in carbon disulphide. It is held to be sensitive down to a level of 0·001%

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(Shenstone & Vickery, 1956) and was used from 1897 as a test for the adulteration of fats with cottonseed oil. Cottonseed oil contains 1–2% cyclopropenoid material, the distribution of which is reported as being 0·7–1·5% malvalic acid and 0·3–0·5% sterculic acid (Shenstone & Vickery, 1961).

Cyclopropenoid fatty acids have been shown to reduce fertility in hens, change the composition of the fat of animals, and cause the deaths of rats. At the same time as we consider these effects in more detail, it should be remembered that a few cyclopropenoid-containing oilseeds, meals and fat are being used as food for man as well as animals (Table 2).

Table 2. *Some cyclopropenoid-containing seeds which are used as food by man or animals*

Species and common name	Use of seed	Cyclopropenoid content of the oil (as % sterculic acid in total fatty acids)	Reference
Gossypium hirsutum; cottonseed	Oil—salad and cooking oil, cooking fats, margarine; Meal—animal feeding-stuff	1–2	Shenstone & Vickery (1961)
Ceiba pentandra; kapok	Feeding-stuff—mainly in Japan	10–14	
Bombacopsis glabra	Reported as being used as famine food in Africa and being eaten in Brazil	35	Pieraerts, Ipatieff & Simar (1928*a*,*b*); de Bruin, Heesterman & Mills (1963); Cornelius *et al.* (1965)
Pachira aquatica		25	

Changes in body fat composition

The body depot fat and abdominal fat of hens fed on a diet containing partially refined and also crude cottonseed oil, have been shown to give a positive Halphen reaction and were, therefore, presumed to contain cyclopropenoid material (Lorenz & Almquist, 1934; Almquist, Lorenz & Burmester, 1934). The deposition of cyclopropenoid compounds in the body fat of hens has been shown to be accompanied by changes in the fatty acid composition of the plasma, liver and ovaries, and to a lesser extent of the heart and depot lipids (Table 3). The stearate content of these fats was shown to increase and the oleate content to decrease on feeding with cyclopropenoid material (Evans, Bandemer, Anderson & Davidson, 1962).

This upset in fat metabolism, which appears to be an impairment of the dehydrogenation step, stearate→oleate, has also been found to be associated with the feeding of cyclopropenoid material to other animals. The depot fat of pigs given rations containing cottonseed meal has been reported to be of higher melting point than normal, owing to an increased stearate and decreased oleate content (Ellis, Rothwell & Pool, 1931; Deuel, 1955). The composition of butter also appears to be affected when cows are given cyclopropenoid material. It has been reported that butterfat from cows given cottonseed meal has a higher melting point, a lower iodine value and a lower volatile acid content than normal (Eckles & Palmer, 1916; Keith, Kuhlman, Weaver

Table 3. *Effect of cyclopropenoid compounds in the diet on the composition of body and tissue fats of hens* (*Evans* et al. 1962)

	Acid			
	Palmitic	Stearic	Oleic	Linoleic
Plasma lipids				
Basal diet	31·3	17·5	34·5	13·7
Basal diet + 2·5% cottonseed oil	33·2	22·0	24·3	18·1
Basal diet + 0·67% *S. foetida* seed (≡0·16% *S. foetida* oil)	32·2	29·0	20·4	16·6
Liver lipids				
Basal diet	23·4	14·0	36·2	22·9
Basal diet + 2·5% cottonseed oil	26·5	22·2	27·7	20·8
Basal diet + 0·67% *S. foetida* seed	24·9	22·4	25·2	24·9
Ovaries and contents				
Basal diet	25·7	10·4	42·4	17·1
Basal diet + 2·5% cottonseed oil	28·5	21·4	26·6	21·2
Basal diet + 0·67% *S. foetida* seed	28·5	23·9	23·0	22·2
Heart lipids				
Basal diet	19·7	9·3	32·3	32·3
Basal diet + 2·5% cottonseed oil	21·4	12·5	30·7	31·6
Basal diet + 0·67% *S. foetida* seed	20·6	13·2	30·5	32·0
Depot fat				
Basal diet	20·6	6·2	36·5	31·5
Basal diet + 2·5% cottonseed oil	23·4	7·9	34·2	29·6
Basal diet + 0·67% *S. foetida* seed	21·0	9·6	43·9	30·0

& Gallup, 1932, 1934). Brown, Stull & Stott (1962) reported a small elevation in the stearate content of milk fat from cows given cottonseed oil with a diet of high roughage content, but with a low roughage content there was no significant effect on the stearate but elevation of the oleate and reduction in the myristate content of the milk fat.

The iodine value of the epididymal fat of rats given *Sterculia foetida* oil has been shown to be markedly reduced compared with that of rats given maize oil (Schneider, 1962).

The supplementation of the diet with oleic acid has been shown to have no effect on the increase in stearate content due to the presence of cyclopropenoid acids, and has little increasing effect on the oleate content of the fat of pullets (Schneider, 1962).

Growth retardation and effect on body organs

Unfortunately, the quantity of cyclopropenoid material given to test animals is expressed in different units by different workers. There are reports that 0·2–0·4% *S. foetida* oil in the diet caused growth depression in chicks (Kemmerer, 1960) and that this oil given at a level equivalent to 66 mg methyl sterculate/lb diet (≡0·03% *S. foetida* oil) did not produce any significant weight difference or mortality in chicks (Deutschman, Berry, Kircher & Sakir, 1964). Growth retardation has been observed in pullets given daily 200 mg *S. foetida* oil but eventually the weight deficiency was made up (Schneider, 1962). This was possibly because the control group of birds were all in egg production after 12 weeks, whereas those given the cyclopropenoid material were sexually immature at that stage.

Growth retardation with 1% *S. foetida* oil and retardation ultimately followed by death with 5% of the oil in the diet of male weanling rats has been observed (Schneider, 1962).

The signs accompanying death of the rats were characteristic of vitamin B complex deficiency, and increasing the quantity of thiamine, riboflavine, pyridoxine, calcium pantothenate and biotin to ten to fifteen times the normal vitamin level of the diet prevented the death of rats receiving 5% *S. foetida* oil. Increased levels of pyridoxine or calcium pantothenate (or both) together with biotin prevented death, but growth was not stimulated significantly by the individual vitamins (Schneider, 1962). Growth retardation on giving kapok-seed oil to 21-day-old rats has been reported (Thomasson, 1955). At a level of 40 cal per cent in the diet over half the rats died and at 60 cal per cent all the animals died at a mean of 14 days after feeding began.

Schneider (1962) observed that when pullets received 200 mg/day of *S. foetida* oil there was no significant difference in liver weight up to 10 weeks after supplementation, but at 19 weeks a marked difference was noted. Significant differences were also noted in the weight of the gall-bladder, ovaries and oviducts at 5 weeks after supplementation and it was observed that comb development was considerably retarded.

The livers of rats fed on a diet containing 1% *S. foetida* oil, and the livers, kidneys and adrenals of rats given cyclopropenoid material at a level of 2·5% *S. foetida* oil were significantly heavier than those of the controls (Schneider, 1962).

Effect on the egg production of hens

As the sexual maturity of pullets given cyclopropenoid material is delayed, the egg production of maturing birds will obviously be affected. But what is the effect of cyclopropenoid fatty acids upon sexually mature hens? Much of the reported data are concerned with cottonseed meal and the results are, therefore, complicated to some extent owing to the presence of the phenolic material gossypol, which may have some depressing effect on egg production (Heywang & Bird, 1954; Narain, Lyman & Couch, 1957).

Marshall & Scott (1948) reported a sharp reduction in egg production and signs of poor health in hens given 5% crude cottonseed oil or 5% *Malva parviflora* seeds; this diet would supply about 20–50 mg/day cyclopropenoid acids. However, Shenstone & Vickery (1959) reported that 25 mg/day malvalic or sterculic acid, or 45 mg malvalic plus 5 mg sterculic acid, had no significant effect on egg production, but that 250 mg sterculic acid caused cessation of laying after 2 days. Similar work carried out by Schneider (1962) showed that *S. foetida* oil at a level of 200 mg/day (≡about 100 mg cyclopropenoid acids) drastically affected egg production, but that production was restored when the feeding of cyclopropenoid material ceased. Schneider (1962) also obtained partial restoration of egg production on giving oleic acid in addition to the *S. foetida* oil, almost complete restoration being achieved at a 3 g/day level.

Effect upon eggs

The stearate content of the yolk fat of eggs from hens receiving cottonseed oil and cyclopropenoid acids has been shown to be higher, and the oleate content lower, than normal (Evans, Bandemer & Davidson, 1960; Evans, Davidson &

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Bandemer, 1961; Evans, Davidson, La Rue & Bandemer, 1963; Schneider, 1962). These changes are mainly associated with the triglyceride fraction and only to a minor extent with the phospholipid fraction (Evans *et al.* 1961).

A large dose of oleic acid, or olive oil, was found to counteract only slightly the effect of cyclopropenoid material in the diet on fatty acid composition (Masson, Vavich, Heywang & Kemmerer, 1957; Doberenz, 1960; Schneider, 1962; Evans *et al.* 1963). The yolk fat of hens given cyclopropenoid material at a level of 9–23 mg/day has been shown to give a positive Halphen test (Lorenz, 1939; Phelps *et al.* 1965). Kratzer, Davis & Marshall (1955) obtained a positive Halphen test with turkey eggs after giving a diet containing cottonseed meal. It has been estimated, from Halphen test response, that about 25% of ingested cyclopropenoid acids is deposited in the yolk of hens' eggs (Shenstone & Vickery, 1959), but this will obviously vary with rate of laying, diet, and other factors.

It should be borne in mind that the Halphen test has been reported to be sensitive, in varying degrees, to a large number of nitrogen-containing compounds and to phosphatides (Bailey, Magne, Pittman & Skau, 1961). On the other hand, because of the difficulties of detection of cyclopropenoid fatty acids by gas–liquid chromatography, failure to obtain a positive result by this technique (e.g. Evans *et al.* 1961) need not necessarily be indicative of the absence of cyclopropene compounds.

A further effect, which accompanies the giving to hens of cyclopropenoid material, is that the whites of eggs produced by these birds become pink on storage. The degree of this disorder increases with the time and temperature of storage (Lorenz, 1939; Heywang, Bird & Altschul, 1955; Shenstone & Vickery, 1959; Deutschman, Reid, Kircher & Kurnick, 1961). The discoloration of the white appears to be due to the formation of a coloured iron–protein complex by diffusion of iron through the vitellin membrane and combination with conalbumin (Bandemer & Schaible, 1946; Azari & Feeney, 1961).

This pink-white effect is accompanied by discoloration of the yolk, to give a salmon, apricot or orange-brown colour. This appears to be due to the migration of the egg-white proteins ovalbumin, conalbumin and lysozyme into the yolk, and the superimposition of the iron–conalbumin colour on the carotenoid pigments of the yolk. The cloudy effect of these yolks may be due to an accumulation of protein from the white (Evans, Bandemer, Davidson, Bauer & Butts, 1954; Evans, Bandemer, Davidson & Schaible, 1957; Evans, Bandemer, Davidson & Bauer, 1959; Shenstone & Vickery, 1959; Kemmerer, Heywang & Vavich, 1961). The lipoprotein livetin migrates to the white from the yolk.

The migration of iron and proteins through the vitellin membrane is accompanied by a transfer of water from white to yolk and an equilibration of the pH of the white and yolk.

Shenstone & Vickery (1956) have suggested that cyclopropenoid acids in the yolk may exert a surface-active effect which is responsible for the migrations through the vitellin membrane, and Kircher (1964) postulates reaction of the cyclopropene material with the sulphydryl groups of proteins, which are closely associated with the lipids, to account for the physiological activity of these compounds.

Embryo mortality

Again, some of the reported data concerned with the effects of cottonseed meal are clouded by the possible effects of gossypol, which has been reported to reduce hatchability of hens' eggs (Heywang & Bird, 1954). However, the use of cottonseed oil and fatty acids isolated from the oil (which would not contain gossypol) indicate that cyclopropenoid fatty acids reduce hatchability. Schneider, Vavich, Kurnick & Kemmerer, 1961) reported that the effect of up to 10 mg/day *S. foetida* oil (≡approx. 5 mg cyclopropenoid fatty acids) in the diet of hens on chicken embryo mortality was not significant, but at five times that level they obtained 77% mortality by the 7th day and 97% by the 19th day of incubation. With 150 mg *S. foetida* oil/day (≡approx. 75 mg cyclopropenoid acids) there was 100% deaths by the 5th–7th day. This work is in close agreement with that of McDonald & Shenstone (1958) who found that sterculic acid given to hens at a rate of 25 mg/day caused 62% infertile eggs after 4 days, 55% of the remaining eggs were dead at 7 days, 94% at 17 days and 100% after 22 days of incubation (control mortality 41% overall).

The effect of cyclopropenoid compounds on embryo mortality and fertility in mice, appears to be vastly different from the effect on chickens. Sterculic acid given at levels up to 200 mg/kg body-weight to male and female mice produced no apparent effect on mating, fertility or mean litter size (Braden & Shenstone, 1958). Deleterious effects are to be found in hens at one-tenth this level of sterculic acid in the diet. It is possible that the difference in effect upon embryo mortality between chickens and mice is connected with the high fat content of egg yolk and the presence of cyclopropenoid compounds in this fat.

Conclusion

The effects itemized above have been shown to be the results of the ingestion of cyclopropenoid compounds. The products obtained by elimination of the cyclopropene ring by hydrogenation, polymerization, halogenation, or reaction with acidic materials, have been shown to give a negative Halphen test response (Kühn & Bengen, 1906; Moore, Richter & Van Arsdel, 1917; Faure, 1956; Masson *et al.* 1957; Nordby, Heywang, Kircher & Kemmerer, 1962) and, in most instances, have failed to have any effect on the fertility of hens or to produce abnormally coloured eggs (Morgan & Ringrose, 1939; Deutschman *et* al. 1961; Nordby *et al.* 1962).

It would, however, be unwise to assume, at this stage, that 'elimination' of Halphen test response and 'inactivation' of cyclopropenoid compounds (as far as the more obvious effects, such as discoloured egg production, are concerned) by chemical methods such as those cited above, is the solution to the problem. Many of the degradation products have yet to be isolated in the pure state and their chemical structure determined, and little work has been done on attempting to ascertain whether or not these products are physiologically active even though they do not produce 'pink whites'.

Naber & Morgan (1957) state that refined cottonseed oil does not depress hatchability, but in that same year Masson *et al.* (1957) observed that a commercial cottonseed salad oil, given to hens, resulted in yolk fat which gave a positive Halphen test, and Harris *et al.* (1964) have detected cyclopropenoid compounds in a refined, bleached, deodorized cottonseed oil. Evans *et al.* (1960, 1962) heated crude cottonseed oil at 150° for 4 h and at 200° for 1 h (conditions which would be expected to polymerize cyclopropenoid acids) and observed that the resultant oil was active as far as pink-white egg production was concerned but inactive in the Halphen test. If cyclopropenoids are to be eliminated from feeding-stuffs by chemical reaction then much work is necessary on the chemical nature and metabolic effect of the reaction products. The most obvious immediate solution to the problem as far as cottonseed (or any other cyclopropene-containing oilseed) meal, as a protein supplement, is concerned, is the solvent extraction of the meal leaving it free from cyclopropenoid material. But what procedure is to be adopted regarding cyclopropenoid-containing fats which are commercially marketed?

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Toxic Contaminants

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Pesticide residues in fat-containing foods and in human fat

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The term 'pesticide' is broadly applied to a wide range of insecticides, fungicides, herbicides, molluscicides, rodenticides and similar substances. Over 100 different agricultural pesticides, of which there are perhaps 800 different formulations, are at present cleared for use in Great Britain. These can be broadly classified according to their general chemical nature into the principal types shown in Table 1. The toxicity to mammals may vary considerably from one pesticide to another within the same chemical type, as indicated in the table. Edson, Sanderson & Noakes (1965) have recently published a useful detailed list of acute toxicities for a large number of pesticides. Safety to both users and consumers in agricultural, veterinary or food storage practice, however, cannot be judged by acute toxicity alone.

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