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3 July, Second Session

Animal Nutrition, Second Part

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The survival of vitamins in feeds containing unsaturated fats

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The practice of adding fats to compound animal feeding-stuffs has not yet become established in this country, although investigations (particularly in Northern Ireland) into its possibilities have been proceeding for some time past. In America, however, where tallow and other fats are easy to obtain, large numbers of experiments have demonstrated that the addition of fat to high-energy rations not only improves the appearance and other physical characteristics of the rations, but also shows economically attractive increases in the weight of livestock feeding on them. The results obtained, however, may not be so advantageous in this country, where the consumer prefers meat and bacon to be much less fat than American tastes demand; added fat in the rations of fattening stock may produce carcasses which are too fat for

consumption in the English ménage. This argument may not, however, apply to poultry, or to dairy animals in which the composition of the milk may be improved.

If highly unsaturated fatty acids are incorporated in a ration at high levels, rancidity tends to develop. When fats become rancid they can become dangerous, either because toxic substances may have been developed or because the vitamins A and E may have been indirectly destroyed. Moreover—and significantly—according to Holman (1960) tocopherol virtually disappears before organoleptically detectable rancidity appears; and vitamin A is destroyed in the early stage of the oxidation of a fat. The destruction of the vitamins by polyunsaturated fatty acids proceeds, according to Dam (1962), before rancidity products accumulate, by interaction of the vitamin with the free radical appearing in the initial stage of the rancidity process. Swift, Rose & Jamieson (1942) had shown that tocopherol is completely destroyed by the end of the induction period. However, Frankel, Evans & Cooney (1959) reported that tocopherol loss during autoxidation was much smaller in the highly unsaturated vegetable oils than in lard. These authors found that metal contaminants appreciably increase the extent of tocopherol oxidation, but that this effect can be eliminated by the addition of 0.01% of citric acid. Dam (1962) remarked that 'In spite of all the information gained to date, the "Interrelations between Vitamin E and Polyunsaturated Fatty Acids in Animals" is still an unfinished chapter'.

Dietary polyunsaturated fatty acids generally accelerate the depletion of, and increase the requirement for, vitamin E, but certain polyunsaturated fatty acids do not produce, or aggravate, all signs of vitamin E deficiency. At least one vitamin E deficiency sign is known to occur only when a certain class of polyunsaturated fatty acids is furnished in the diet: for instance, encephalomalacia in chicks appears when the diet contains linoleic or arachidonic acid, but not when the diet is fat-free or contains linolenic acid. Hill (1963) showed that, for every 1% of peroxidized maize oil added to the diet above 4%, 100 mg D- α -tocopheryl acetate are necessary to protect pigs from erythrocyte haemolysis. Weber, Weiser & Wiss (1964) have shown that linoleic acid reduces the uptake of DL- α -tocopherol from the intestine, and that there is an increase in the requirement for vitamin E in the rat of about 0.5 mg DL- α -tocopheryl acetate/g linoleic acid. Weber, Gloor & Wiss (1962), using radioactive vitamin E, had observed that, in both man and animals, there was an increase in the requirement for vitamin E in the presence of the polyunsaturated fatty acids, and they raised the question of how far the natural vitamin E content of edible fats was sufficient to meet this increased need. On the other hand, several manifestations of vitamin E deficiency may occur with suitable diets even when the diet does not contain polyunsaturated fatty acids.

When fats are used in 'milk replacers' of 'calf starter' rations (to replace the milk fat removed for butter and cream making) the level is low, about 2½% representing the limit above which palatability is adversely affected. It has been claimed that the addition of fat to the ration of steers increases energy intake, and improves performance and feed utilization; but the quality of the roughage influences the effect of the added fat, gains in weight rising in proportion to the quality of the roughage. The fat added to 'beef' rations rarely exceeds 5% of the total ration or about 0.5 lb

fat per animal daily. Bohman has recently been reported (Anonymous, 1965) as saying that 'the optimum level, with present knowledge, appears to be between 3-5%. The relation of the cost of animal fat with appropriate substitute energy sources may ultimately be the primary factor in determining the appropriate level of use'.

When fat is added to animal feeding-stuffs the resultant total fat content of the complete ration includes the added fat, which may be a tallow, or a vegetable oil, and the lipids naturally present in the major components of the feeds, which may include maize, and fish or cottonseed meals. These lipids will be more sensitive to oxidation since they contain a larger proportion of unsaturated fatty acids.

Blain & Shearer (1963) have suggested that in some circumstances the stability of vitamin A and carotene in feeding-stuffs may be dissociated from fat oxidation, but state that it would be artificial on the whole to divorce the two topics. There has been a growing practice to add antioxidants to feeding-stuffs with the dual intent of protecting both the vitamins and the fats. Siedler, Enzer, Schweigert & Riemenschneider (1956) showed that, when antioxidants were used with added fats in feeds, the protection of the fats did not correlate with that of vitamin A or carotene; antioxidants which were effective for the one, were poor protection for the other. Recent unpublished chemical assays by Roche Products Ltd on a number of feed supplements have shown that the stability of the vitamin A present in the form of the gelatine beadlet is not improved by the presence of ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) as a component of the compound feed. Nevertheless, the manufacturer feels himself still justified to include the antioxidant, but only to protect the fats present as such. Recently Privett (1962) showed that the use of antioxidants has been entirely empirical.

Zimmerman & Wostmann (1963) have investigated the stability of the vitamins in three diets which were subjected to sterilization before being given to germ-free animals. The process of sterilization consisted of 20 min under reduced pressure at room temperature followed by 25 min at 123° under 17 lb of steam pressure. The pH of the diet was 6.1, and a full range of minerals including copper and iron was present. In one of the three diets 5% maize oil was present. The mean retention of the vitamins was: riboflavine, 95%; vitamin B₆, 80%; thiamine, 65%; calcium pantothenate, 92%.

These results depended on the presence of a small amount of added water, but Zimmerman & Wostmann observed that 'the mechanism by which the addition of water protects some of the B vitamins is as yet not known'. Similarly, the retention of vitamin A even under these severe conditions was of the order of 85%.

When antioxidants are used, they may be added to the fats before the compound feed is formulated, or may be included as a separate component amongst the major ingredients of the ration. If added to the fats thus, they are effective only if the fat has been recently extracted and hence is free from incipient rancidity. Moreover, the major constituents, such as maize or fish meals, must also be fresh, because if they have already been held in store, and rancidity has begun to develop, the need to raise the level of the antioxidant becomes imperative; even so, the effect of antioxidants

can be nullified altogether. Contact with metals during the handling, storage and transport of fats in tanks, drums and pipes will diminish the effectiveness of antioxidants. All the metals in common use, copper, nickel, cobalt, manganese, and even zinc and iron, are deleterious; only aluminium and lead are inactive. Hence, to protect the protection, it is usually necessary to incorporate sequestering agents such as citric acid, tartaric acid or EDTA.

Chemical assays recently completed by Roche Products Ltd on feeding-stuffs containing antioxidants, and compared with corresponding assays on controls without antioxidants, have failed to show any significant differences in the survival of vitamin A added in the form of the gelatine beadlet.

There would appear to be two aspects of rancid fats in feeding-stuffs: the gross dietary effect, and the effect of the peroxides produced on the vitamins. Niesar (1962) showed that poultry (unlike rats) make relatively good use of rancid fats; he found that, provided the fat in the diet did not exceed 10%, no adverse effects appeared. However, when rancid fats were given, the requirements for vitamin E increased, as shown by the reduced storage of vitamin E, particularly in the heart and the liver.

From this it follows that fats which are not entirely fresh may indeed be included in poultry fattening diets, but care must be taken to ensure an adequate supply of vitamin E. Niesar concluded that fats with peroxide values of up to 10, aldehyde values up to 1, and acid values up to 20 are tolerated without difficulty in pigs and calves as well as poultry, provided that they do not contain dangerous levels of polymerization products.

Hence, to recapitulate, fats are being used in feeds; even partially rancid fats may be used in feeds; antioxidants, whether protected with sequestering agents or not, are not entirely effective in preventing developing rancidity, with the consequent hazards to the oxygen-sensitive vitamins.

It was reflective thinking of this kind which led to the development of the so-called gelatine 'beadlet' by Hoffmann—La Roche & Co., Basle, Switzerland. Theoretically, the fat-soluble vitamins should be so contained within compounded feeding-stuffs that they would be capable of surviving the onslaught of peroxides, ketones and aldehydes during the manufacture and subsequent storage of the feed, and yet be readily capable of dissolution in the intestine and stomach of the animal consuming the feed.

After prolonged and intensive examination by Roche of many possible materials, gelatine, allied with sugar to facilitate dissolution in aqueous media, appeared to offer most promise, but gelatine with many differences. In the early days, the specifications presented to the gelatine manufacturers astonished them: these specifications required certain properties which had been traditionally held in the industry to be mutually exclusive: low viscosity, high jelly strength and extreme purity. In so far as they could be met at all, only the meanest kind of gelatine seemed at all likely to conform.

After considerable consultation, experimentation and adaptation, British Glues and Chemicals, employing an entirely unique process of extraction from a most unusual source of the raw material, evolved a gelatine which approximated to the

specifications: ideally, it should be entirely resistant to the action of water and heat, changing pH and pressures, and chemical environment when in the compound feed before consumption; yet it must rapidly disintegrate when contained in the gastric juices. The gelatine now employed in the beadlets is, of its kind, something of an oddity; yet, under most practical conditions, it ensures that the fat-soluble vitamins reach the animal without loss, and become entirely available when in the digestive tract.

Other physical characteristics of the beadlet are of almost equal importance. There are some 100 000 beadlets in 1 g, and they measure about 0.2–0.4 mm in diameter. Hence these beadlets approximate very closely to the average particle size occurring in most conventional feeds. The specific gravity of the beadlets is about 0.7, which is well within the range of densities of the other conventional components of feeding-stuffs. This means that the possibility of segregation is reduced to a minimum, an advantage which is still further ensured by the rough surface of the beadlets, which tends to make them cling to the other particles surrounding them even under the vibration and agitation of conventional manufacture and transport.

The gelatine is resistant to cold water, but tends to disperse readily, quickly and completely in warm water at 80°–90° or in aqueous menstrua of any kind. Within the beadlet, the vitamin, whether A, D or E, is finely distributed throughout the solidified matrix. The individual globules of the oily vitamins scattered throughout the gelatine matrix measure about 1–4 μ m in diameter, with the majority nearer the lower figure, so that the passage through membranes is almost as free as if the vitamins were in true solution. Within the ivory tower of this gelatine beadlet, there is a second inner line of defence consisting of antioxidants, including free α -tocopherol, which reduces the effect of any entrapped oxygen in the system.

The superiority of the gelatine beadlet was conclusively demonstrated by Olsen, Harvey, Hill & Branion (1959), who reported that, judging by liver storage of vitamin A, gelatine-coated preparations were superior to preparations in which the vitamin was coated with wax or fat, or was adsorbed to vegetable protein. Feeding oils and dehydrated cereal grass were the poorest sources. The data from stability tests indicate some deterioration of the vitamin in all preparations and, although definite conclusions were difficult to draw, the superiority of gelatine preparations was indicated.

In the 6 years since this Canadian report appeared, the performance of the beadlet has been still further improved.

The composition of the gelatine matrix is under constant test, and many scores of different gelatines have been scrupulously examined in the search for still better protection. Though, generally speaking, routine assays on feeding-stuffs show losses of vitamin A of the order of 5%–10% after periods of storage commonly encountered in the trade, results are occasionally obtained which are surprisingly poor. Many supplements contain choline chloride. There is some evidence—though this is far from being conclusive—that vitamin A is unstable in the presence of choline chloride. A case recently encountered by Roche produced reports of

serious losses of vitamin A in a new feed supplement, having a fairly conventional composition, which had been subjected to an accelerated storage test. This test consists of holding the preparation containing the active substance at a temperature of 45° for 4 weeks. The loss of vitamin A under these conditions very roughly approximates to the loss to be expected after about 1 year at room temperature. In this particular instance choline was present. A closely similar preparation, also containing choline but at a lower level, had shown quite reasonable retention of vitamin A.

The explanation was found after we realized that the choline chloride normally used in feeding-stuffs is an 80% solution in water. It appeared that the addition to the supplement of the somewhat higher level of choline chloride brought with it enough water to reach what is presumably a critical threshold of water; it was sufficient, at the elevated temperature of the test, to attack the gelatine and so expose the vitamin to the disruptive influences contained in the mixed feed. This theory was afterwards substantially confirmed when it was subsequently discovered that samples of the same supplement, containing the same amount of choline chloride, and hence of water, which had been stored at ordinary room temperature, showed satisfactory retention of the vitamin. This is, therefore, an example of the system of measurement being at fault—a salutary reminder that, when assay results prove unsatisfactory, one must suspect not only the sample, but also the method of examination.

Conclusion and summary

The normal hazards—and consequent failings—of the natural sources of the vitamins, and especially of vitamin E, have induced the search for a form of the vitamin which could be confidently expected to survive in animal feeding-stuffs. The great sensitivity of the vitamin E from natural sources, for example, arises from the fact that it is usually present as the free phenol. The international standard for vitamin E is the acetate, which is very much more stable; it is entirely devoid of the antioxidant properties which some think, contrary to the balance of available evidence, is the only function of the vitamin in the body. The form of the vitamin now almost universally used in feeding-stuffs is the gelatine beadlet containing the stable acetate—which, nevertheless, is still provided with the excellent additional physical barrier of the gelatine. Vitamin E added to feeding-stuffs in this form is remarkably stable.

Manufacturers (or compounders) now tend to ignore all of the naturally occurring vitamins, which may or may not still be present in compounded feeds after manufacture and storage, and to base any claims for biological potency only on the pure substances added as separate components to the feeds.

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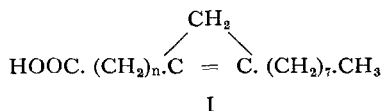
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Adverse effects of cyclopropenoid fatty acids

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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.



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
<i>Sterculia macrophylla</i> (seed)	63	4	} F. D. Gunstone (1964), unpublished work Wilson, Smith & Mikolajczak (1961) Earle, Melvin, Mason, Van Etten, Wolff & Jones (1959); Harris, Magne & Skau (1963) Cornelius, Hammonds & Shone (1965)
<i>S. parviflora</i> (seed)	45	19	
<i>S. foetida</i> (seed)	55	7	
<i>S. foetida</i> (seed)	Total 44-45.6		
<i>Bombacopsis glabra</i> (seed)	35	Nil (< 0.5)	

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%