RAPESEED MEAL AND EGG TAINT

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Rapeseed as a source of oil and protein is expanding rapidly, especially in Europe. The development of "double zero" varieties with low levels of erucic acid and glucosinolates is being extended to include reduction of linoleic acid content and the thickness of the outer seed coat. Rapeseed meal from "double zero" varieties has proved to be an excellent source of protein for farm animals but its use in rations for laying hens is still restricted, like all other rapeseed meals, because, when it is fed to certain brown egg laying birds, eggs with a "crabby" or "fishy" taste are produced. In most European countries where over 50% of the production is of brown eggs, and the ratio of brown/white is increasing, the use of rapeseed meal in layer rations is minimal.

In the UK, large scale tainting of eggs was first encountered in 1971-2. The problem was quickly related to the inclusion of rapeseed meal in the layer rations and it was shown that only a proportion of the brown egg layers of major commercial flocks was susceptible; commercial flocks of white egg layers were not significantly affected. Rapeseed meal was withdrawn from poultry rations in the UK and has not been used in these rations since that time.

Large numbers of eggs from affected flocks were examined by sensory assessment and the volatile material from tainted eggs was collected. Subsequent analysis showed that the "crabby" odour could be directly correlated with the presence of trimethylamine (TMA) in the whole egg at concentrations of 1µg/g or greater. This level was adopted to define a "fishy" egg. However, free TMA does not normally occur in rapeseed meal. Furthermore, the ubiquitous occurrence of this chemical as part of the normal metabolic processes in higher animals made investigation difficult. A further complication arose from the fact that within susceptible (i.e. tainting) strains only certain birds were affected by rapeseed meal. About 10 % of "tainters" were usually observed in commercial flocks. This selectivity (Vondell, 1949; Vogt, 1964) suggests a genetic element which has recently been studied (Bolton et al., 1976).

The factor(s) involved in the tainting process have been investigated using chemical fractionation and bioassays based upon ability of susceptible hens to transfer increased levels of TMA (>0.5µg/g egg) to the egg when fed diets containing these tainting substance(s).

The analysis involves sublimation of TMA from frozen (-198°) whole egg under vacuum (0.1mm Hg) in a cold finger distillation apparatus. The frozen distillate is then assayed for TMA by gas chromatography (lower detection limit of 0.05µg/g egg). Recent improvements, including the use of a nitrogen detector, have decreased this limit to 0.01µg/g. Using this method, changes in TMA levels in eggs from selected "tainters" changed from control diet to rapeseed meal, and vice versa, can be measured (Hobson-Frohock et al., 1975).

We have found that all rapeseed meals which we have examined cause comparable increases in the levels of TMA in eggs. These meals have

included Altona, Span P, Yellow Sarson and the low glucosinolate variety, Bronowski. The latter finding strongly suggested that the glucosinolate content was not related to taint induction and this was further confirmed by feeding myrosinase-treated diets, which were rich in 5-vinyloxazolidine-2-thione (OZT, goitrin) or hydroxynitriles, and finding no appreciable reduction in the levels of TMA in the eggs. More recently the two "double-zero" cultivars Tower and Erglu have been shown to induce taint (Hobson-Frohock et al., 1977; Vogt, 1976). The closely related oilseed Crambe Abyssinica causes tainting, and there are also reports of tainting following the feeding of mustard meal.

The following fractionation scheme, in which active fractions are those capable of causing taint, was developed. Rapeseed meal was defatted, extracted with 11% aqueous acetone and the freeze-dried active extract (6% of original meal) was then partitioned between aqueous methanol and chloroform to yield an active polar fraction (5%). After passing this fraction through Amberlite IR-120(H+) cation exchange resin the freeze-dried effluent (3.5%) was found to be inactive.

The chemical components of rapeseed meal which would be absorbed on such a resin include free and bound choline derivatives. In rapeseed meal and many other brassicas, the major form of bound choline is sinapine, the choline ester of sinapic acid, present in rapeseed to the level of 0.6 - 1.5%. The ion-exchange effluent was therefore supplemented with sinapine bisulphate (prepared from white mustard flour) and this restored the original activity. Surprisingly, feeding sinapine bisulphate alone gave a response in only a small number of birds. When a sample possessing 97% by wt. of the original meal, but only 10% of the original sinapine, was prepared by recombination of the inactive fractions from the preparation described and this material was fed, both alone and supplemented with sinapine, the effect of sinapine was confirmed. Sinapine is thus an important element in the production of tainted eggs but is probably not the only factor involved. Studies are continuing to isolate and identify these other factor(s).

The decrease in the level of tainting after reduction of the sinapine content of the meal suggests that the problem may be overcome by breeding "zero" sinapine lines or by chemically reducing the levels of sinapine in existing cultivars. We have examined the sinapine content of over 130 seed samples and found none outside the recorded range of 0.6 - 1.5%. Although Tower (0.7%), Candle (0.6%) and Crambe Abyssinica (0.4%) meals are much lower than average, they also cause taint when fed.

We have also looked at methods of reducing (or removing) sinapine from commercial rapeseed meals. Treatment of the meal with 4% aqueous $Ca(OH)_2$ reduces the sinapine level to about 5% of its original value and when this treated meal is fed it produces much lower levels of TMA in eggs. A similar reduction in sinapine content occurs on ammoniation of rapeseed meals.

Finally, in view of the apparent genetic involvement in the birds, it is possible that a third approach to the problem might be through appropriate breeding programmes. However, this requires a better understanding of the basic biochemistry and physiology of the birds which are susceptible to rapeseed meal. Collaborative investigations with Houghton Poultry Research Station have shown that there is a substantial difference between tainters and non-tainters in their ability to metabolise TMA to TMA-oxide. This is reflected in the rate of disappearance

of TMA injected into the bloodstream and in the activity of the TMA-oxidase enzyme in the liver of tainters. In vitro tests show sinapine to be a potent inhibitor of this enzyme.

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