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Deleterious Substances in Animal Feedingstuffs

Summaries of two papers given at an ordinary meeting of the Association of Public Analysts on 20 October 1976 at the School of Pharmacy, 29/39 Brunswick Square, London WC1 1AX.

**THE DETECTION AND DETERMINATION OF THE
UNDESIRABLE VEGETABLE SUBSTANCES SPECIFIED IN
SCHEDULE 3 OF THE FERTILISERS AND FEEDINGSTUFFS
(AMENDMENT) REGULATIONS, 1976**

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The table of prescribed limits for undesirable substances in feedingstuffs which appears in the Fertilisers and Feedingstuffs (Amendment) Regulations 1976¹ involves Analysts in the detection and determination of the various vegetable impurities included in Schedule 3.

The author is mainly concerned at this stage with methods of analysis. The merits of the limits which have been laid down are secondary. It should, however, be pointed out that some of the substances included in the Schedule have at one time or another caused the death of farm stock and also have, in the past, been the subject of disputes in international trade.

The determination of the undesirable vegetable substances specified in the schedule will probably introduce a new type of analysis to many agricultural analysts. The techniques employed are quite different from normal analytical processes which make use of precipitations, titrations or one of the many modern instrumental methods. They involve "hand picking" of a prepared sample.

The essence of this process is in preparing the sample for picking and in confirming the identity of the material picked out. There are no sensitive colorimetric reactions which enable a few parts per million of a contaminant to be determined in a sample of 5 g, so that to attain the low limits specified we have usually to work on a very large sample. Microscopy plays an important part in many aspects of the work.

Sampling

Before considering individual substances it should be pointed out that the introduction of undesirable vegetable substances in Schedule 3 of the 1976

Regulations means that the directions for sampling in the 1973 Regulations² need to be substantially modified.

As the Regulations stand, a sample of as little as half a pound may form the official portion submitted to the agricultural analyst; such a portion is quite inadequate since it is the amount required for one castor test only.

If a meaningful examination is to be made in respect of these undesirable vegetable substances, at least a three pound sample should be submitted to the analyst. Also, the directions for the preparation of the sample need to be modified. The basic requirement of the method of preparation as laid down is that the sample needs to be passed through a 2-3 mm sieve before a final small portion is finely ground for analysis.

Such a procedure would largely spoil any results of analysis subsequently attempted for some of the deleterious substances. Clearly, unground cereals which are to be examined for ergot should be left in their original condition; the fine grinding of a sample prior to the determination of castor will render the final picking over an extremely tedious task, and it would make the determination of weedseeds and uncrushed fruit containing alkaloids, etc., a complete waste of time.

Basically, the author's advice on sampling is that at least three pounds of sample should be submitted to the analyst and that only $\frac{1}{6}$ th or half a pound of that should be prepared according to the Regulations.

TABLE I
DIVISION OF 3 LB SAMPLE

Sample portion	Sieve used in preparation	Tests on portion
2/6th	Left in original state	} <i>Datura stramonium</i> , <i>Lolium temulentum</i> , <i>Lolium remotum</i> and other whole weed seeds and fruits containing alkaloids and glucosides etc., crotalaria, ergot
3/6th	$\frac{1}{4}$ inch	
1/6th	1 mm	} Lobelia Count. Brassica, camelina etc., volatile oil of mustard, hydrocyanic acid etc.

Analysis for Some of the Undesirable Vegetable Substances

ERGOT

Ergot consists of the sclerotium or fruiting body of the fungus *Claviceps purpurea* and, as well as its occurrence in rye which caused so much trouble in the Middle Ages, it is now also found in wheat, particularly French wheat.

The process of analysis for ergot is simplified by the fact that the schedule limits ergot only in unground cereals in which it may easily be picked out. For this purpose, two aliquot portions of about 200 g each are taken from the sample and the pieces of ergot are picked out by hand. In some cases it is found that a slotted sieve can be helpful. With a bold cereal the ergot will drop through the slot and the bulk of the cereal will remain on the screen.

The analysis is not always a simple one, since in some cases the grain may be only partially ergotised and these grains must be picked out and the proportion of ergot in them taken into account when assessing toxicity.

In the case of samples of ground feedingstuffs, such as meals, it is possible to identify ergot under a lower power microscope. Alternatively, an attempt may be made to determine the presence of the ergot alkaloids, using the dimethyl-amino-benzaldehyde colour test, although it is my experience that with feedingstuffs in the ground condition, the alkaloids may decompose with time.

The limit for ergot in unground cereals laid down in the Regulations is one which has been generally accepted and, moreover, some milling authorities say that this proportion is also acceptable in flour for human consumption.

It is odd that the Regulations apparently are not concerned with compound meals prepared from cereals which contain a high proportion of ergot.

CASTOR, CROTON AND CURCAS

These three undesirable substances are best considered together since the method of isolation of the seed husks of all three is the same and it is only in the final stages of the analysis that they are differentiated. In practice we are usually only concerned with castor since the other seeds are very rarely encountered. During the past century, castor has caused more trouble than any of the other deleterious substances and, therefore, the author has given it special consideration.

It will be appreciated that the poisonous constituents of these three seeds are present in their fleshy endosperms and not in the husks, but, as the active constituents are almost impossible to detect chemically at low levels, reliance must be placed on the isolation of husk.

The detection and determination of the husks is made possible by two properties which all three seeds have in common:

- (1) the dark husk or testa is not decolourised on treatment with bleaching powder;
- (2) the husks are heavier than the majority of other seed testas.

Preparation of the sample before commencing an analysis for castor seed is restricted to passing it through a quarter inch sieve, the coarser parts being gently crushed until they pass through the sieve.

The method of isolation consists essentially of making fibre preparations from two 250 g portions of the feeding stuff, the operations being carried out in plastic buckets and the heating effected by passing live steam into the suspension of the feed first in dilute acid and secondly in alkali.

The washing operations are carried out by decantation and during both of these manipulations the lighter pieces of husk from other seeds and grains in the mixture will be largely removed by levigation. The final stage in the seed fibre preparation consists of treatment with bleaching powder and then washing.

The bleached fibres which remain in the bucket are washed out into a 12 in. × 18 in. photographic dish and the pieces of hard black husk are then picked out. It is important that the pieces of testa which are selected should be hard. From

time to time, very brittle pieces of black material are encountered which are not any of the three husks under consideration. These pieces differ, however, because they disintegrate very easily under gentle pressure from a pair of forceps.

The pieces of black testa which have been picked out are finally dried at 100°C and weighed; the testa loses a small percentage of its weight as the result of the foregoing drastic treatment and so we must multiply by 1.2 to give a truer estimate of the amount of original husk. This factor has been arrived at as a result of examination of seeds from six widely separated parts of the world.

The final stage in the analysis is to confirm the nature of the pieces of husk which have been picked out. In most cases these will consist of castor, and the confirmation is made microscopically. First, the husk is examined with a low power microscope and the characteristic pitted or mottled surface with very serrated edges should be found. This alone, however, is not sufficient to establish the presence of castor, since the testas of all the three seeds are similar when seen under low power magnification and fragments of grapeseed are also very similar. It should be remembered that grapeseed residues are occasionally legitimately used in compound feedingstuffs; grape husk behaves similarly to castor in the test and can be a trap for the unwary.

For final identification it is necessary to examine the individual fibres of the testa under high power magnification, and for this purpose the testas are boiled in a small beaker with 10 ml of 20 per cent. nitric acid until the husk softens and becomes yellowish in colour. The softened husk is then mounted and examined under the high power objective of the microscope; the differences in structure of the different seeds have been described by F. Robertson Dodd³.

It is very easy to distinguish between castor and grape. The fibres of the latter are much shorter. Differentiation between castor, croton and curcas, however, requires careful further examination of the inner structure of the fibres.

Occasionally, it is necessary to examine grapeseed meals and cakes for castor. For this exercise a more drastic bleaching process must be employed in order to reduce the colour of the grapeseed husk. This can be achieved either by the use of a stronger bleach solution or, alternatively, by the use of hydrosulphite solution after the treatment with bleach.

The limit laid down in the Regulations for castor husk is 10 milligrams per kilo and this limit applies to all feedingstuffs. Analysts who are not familiar with the determination of castor will therefore appreciate why it is necessary to employ such a large amount of the sample for the analysis.

Even when working on a portion of 250 g of the sample the maximum limit only amounts to the presence of 2 mg of castor husk, and since the husk is sometimes badly distributed, it is vital in analysis that two portions of 250 g should be examined. In the main, agreement between duplicate portions can be expected to be very good, even at the low levels of 10 and 20 milligrams per kilo. However, if one of the portions is found to contain castor while the other is free from it, then, unless the amount of castor found in the one portion is trivial, i.e. less than a milligram, it is usually advisable to start the examination again with two new portions of 250 g.

In view of the importance that castor has assumed in commercial transactions during the last half century and the future possible implications under the

TABLE II
CASTOR HUSK 1-20 MG PER KILO

Sample	Husk in 250 g duplicates, <i>mg</i>	Equivalent to <i>mg per kilo</i>	Mean, <i>mg per kilo</i>	Difference from mean, <i>mg per kilo</i>
152	3	14.4	16.8	2.4
	4	19.2		
2917	0	0.0	1.2	1.2
	0.5	2.4		
2444	1	4.8	4.8	nil
	1	4.8		
956	2	9.6	12.0	2.4
	3	14.4		
951	3	14.4	16.8	2.4
	4	19.2		
952	2	9.6	12.0	2.4
	3	14.4		
953	1	4.8	9.6	4.8
	3	14.4		
543	1	4.8	7.2	2.4
	2	9.6		
3886	0	0.0	2.4	2.4
	1	4.8		
2911	0.5	2.4	2.4	nil
	0.5	2.4		
2913	2	9.6	4.8	4.8
	0	0.0		
240	2	9.6	9.6	nil
	2	9.6		

TABLE III
CASTOR HUSK 21-80 MG PER KILO

Sample	Husk in 250 g duplicates, <i>mg</i>	Equivalent to, <i>mg per kilo</i>	Mean, <i>mg per kilo</i>	Difference from mean, <i>mg per kilo</i>
50	4	19.2	21.6	2.4
	5	24.0		
106	5	24.0	24.0	nil
	5	24.0		
2920	9	43.2	40.8	2.4
	8	38.4		
5134	8	38.4	38.4	nil
	8	38.4		
3591	12	57.6	55.2	2.4
	11	52.8		
2000	12	57.6	50.4	7.2
	9	43.2		
5134	8	38.4	38.4	nil
	8	38.4		
233	10	48.0	43.2	4.8
	8	38.4		
2325	13	62.4	67.2	4.8
	15	72.0		
105	12	57.6	64.8	7.2
	15	72.0		

TABLE IV
CASTOR HUSK 81-200 MG PER KILO

Sample	Husk in 250 g duplicates, mg	Equivalent to, mg per kilo	Mean, mg per kilo	Difference from mean, mg per kilo
204	17	81.6	81.6	nil
	17	81.6		
1411	19	91.2	91.2	nil
	19	91.2		
2543	20	96.0	100.8	4.8
	22	105.6		
1431	34	163.2	156.0	7.2
	31	148.8		
3005	19	91.2	103.4	12.2
	24	115.2		
1848	20	96.0	100.8	4.8
	22	105.6		
2549	22	105.6	110.4	4.8
	24	115.2		
2586	25	120.0	110.4	9.6
	21	100.8		
3105	40	192.0	199.2	7.2
	43	206.4		
5839	27	129.6	136.8	7.2
	30	144.0		
104	40	192.0	204.0	12.0
	45	216.0		
1501	25	120.0	124.8	4.8
	27	129.6		

Fertiliser and Feeding Stuffs Act, some of the results which have been obtained over a period of several years have been recorded in Tables II, III and IV, and they illustrate the usual agreement that can be obtained when working with duplicate portions of 250 g. The tables show the results of duplicate assays and also the deviation of the individual figures from the mean.

From the point of view of the Act the figures shown in Table II are the most important since they relate to the maximum allowable level of 10 milligrams per kilo. The results are good, bearing in mind the great difficulty of obtaining an even distribution of such small amounts of castor husk. Nevertheless, they emphasise the need to exercise caution before condemning a feedingstuff which may be found to have a mean content of castor which is just outside the prescribed limit.

Since this is the first time that the results obtained in duplicate castor husk determinations have been published, Table V provides a very simple statistical

TABLE V
STATISTICAL ASSESSMENT OF RESULTS IN DIFFERENT GROUPS

Group castor husk, mg/kilo	Standard deviation of differences from means, mg/kilo	Standard deviation of differences from mean expressed as percentages of means
1-20	2.2	40
21-80	3.1	19
81-200	8.8	11
201-1000	26.4	13
1001-10,000	103.0	8

assessment of the results in the different groups, based on different levels of castor husk.

The table includes the standard deviation from the means of the individual results obtained in duplicate determinations and also shows the standard deviation of the differences from mean figures when calculated as percentages of the mean.

Table V includes a number of results which are not given in Tables II, III and IV and also includes two additional groups relating to larger quantities of castor husk.

The results not only serve to emphasise the need for caution in condemning samples which may be slightly in excess of the limit of 10 milligrams per kilo, but they also indicate how the accuracy of the determination increases at the higher levels of castor in spite of the inaccuracies which could be expected to result from the tedium of picking out larger numbers of fragments of castor husk.

"No Detectable Amount" Group

We can now consider that group of undesirable substances for which the permitted content is "less than the lowest detectable level". This virtually amounts to a "zero tolerance", a tolerance which resulted in trouble and argument in the U.S.A., both with lead residues and more recently in the case of pesticides. Public Analysts might profitably seek to amend this specification, if only by initiating discussions with other interested parties.

Croton and curcas are included in this group and their determination has already been discussed.

A general limit of 10 milligrams per kilo for croton is reasonable but the author would like to see a much lower limit for curcas.

OBJECTIONABLE BRASSICA

This group of undesirable seeds includes several varieties of brassica and the Regulations prohibit there being any detectable amount present. They may be present as whole weed seeds in whole cereals or as small fragments in cakes and compounds. Whole brassica seeds, when they occur, may be picked out and finally identified by a microscopical examination of the testas.

The method of detection and estimation of prohibited varieties of brassica in compound feedingstuffs or cakes follows the method already described by the author in 1966⁴ for the detection and estimation of vegetable impurities in feedingstuffs. For this determination, the finely ground sample (passing a 1 mm sieve) is employed, and a 0.90 g portion, obtained by a careful quartering of this sample, is added to 0.10 g of lobelia seeds. A micro preparation is prepared by boiling alternately with acid and with alkali, care being taken to avoid any loss of particulate matter during filtering operations (a nylon cloth is an asset).

The prepared fibre should be transferred to a white tile and quartered down while wet, in order to obtain the portions to be used for examination. The fibre obtained in this manner is usually sufficient for four test slides. After mounting the fibre in mucilage of tragacanth, the characteristic structures are counted

using a 1 in. objective. The number of lobelia seeds and the number of squares occupied by the determinant are counted in each field.

Counting 60 fields on each slide is usually convenient, the fields being equally spaced with the aid of a mechanical substage. When following this procedure, it is possible to detect 0.2 per cent. of *Brassica nigra*.

The order of counts which are obtained are as follows:

Lobelia (seeds)	Brassica (squares)
157	12
130	8
157	14
135	10

The results indicate that there would be little difficulty in detecting 0.1 per cent. of *Brassica nigra*, but the percentage of Chinese yellow mustard *Brassica juncea* (L.) (Czern & Coss) which similar counts might represent would be higher because this particular rape seed yields a smaller proportion of outer testa.

The testa of many other seeds, including some other undesirable ones, may if required also be counted at the same time.

The presence of the undesirable species of brassica is usually related to the proportion of volatile mustard oil; or at least it should be in practice, but it would appear from the Regulations that this is not recognised.

The explanation of the anomaly is that the volatile oil in the prohibited species is mainly in the form of allyl isothiocyanate while in the other species the less toxic butenyl compound predominates.

Mention should be made of the method prescribed in the 1976 Regulations for the determination of volatile oil of mustard. The prescribed method is reasonably accurate for quantities in excess of 600 mg per kilo, but below this level the strength of the volumetric silver nitrate and ammonium thiocyanate solutions should be substantially reduced. At volatile oil levels below 50 mg per kilo a colorimetric finish is to be preferred.

The following results which have been obtained with mixtures of *Brassica nigra* and white mustard illustrate the point:

<i>Brassica nigra</i> , per cent.	Volatile oil of mustard, per cent.	Normality of AgNO ₃	Theory V.O.M., per cent.
100	0.930	0.1	0.93
5	0.042	0.1	0.047
2	0.019	0.02	0.019
0.5	0.006	0.02	0.0047

The results show that at levels of 400 mg per kilo the accuracy of the test diminishes. This is due to the very small titre figures obtained. If the normality of the titrants is substantially reduced, however, a considerable improvement in reproducibility is obtained, but of course, such a procedure is departing from the requirements of the Regulations.

The author's experience in this field of agricultural analysis leads him to state categorically that the zero tolerance is quite unsuitable for brassicae.

Most botanical authorities agree that of all the many genera in the agricultural plant kingdom the one in which specimens are most likely to be "mixed up" is brassica, as anybody who has tried to obtain a pure specimen of a particular species will already know.

On many occasions, the author has found traces of prohibited species of brassica in various animal feedingstuffs, and has disregarded them as being insignificant. Today, this could cause difficulties. The author would suggest that the limit for the different prohibited species of brassica should be kept in line with the volatile oil of mustard limit and set at about 2 per cent.

APRICOT AND BITTER ALMOND

Apricot and bitter almond are presumably included in this group on account of their content of cyanogenetic glucosides and the author would have thought that general limits for hydrocyanic acid would have sufficed. Methods for the detection of the seeds undoubtedly lack specificity and would be less sensitive.

CAMELINA SATIVA

Camelina sativa is a small pale brownish seed. Its prepared fibre is very characteristic, enabling it to be counted by the lobelia method.

BASSIA, MADHUCA LONGIFOLIA AND MADHUCA LATIFOLIA

Bassia, in the author's experience has presented a difficult problem. Both species have fairly large seeds with thin testas and these have useful microscopical characteristics which may be employed for diagnostic purposes, but the endosperm has few distinctive microscopical characteristics to help in its detection in a mixture. Unfortunately, most commercial samples of mowrah (madhuca) meal are almost free from husk so other methods of detection must be sought. Three methods have been suggested:

- (1) a colour test⁵;
- (2) separation of the saponin-mowrin⁶;
- (3) a biological test using worms as the test subject.

Weedseeds and Unground, Uncrushed Fruits Containing Alkaloids etc.

The three seeds which are specifically mentioned, together with limits for them are *Datura stramonium* (thornapple), *Lolium temulentum* and *Lolium remotum*.

The detection in feedingstuffs of whole undesirable seeds such as thornapple may present a difficult problem because the seeds tend to become coated with the mealy portions of the feed, thus obscuring their true identity. The acid fibre technique overcomes this difficulty.

Acid Fibre Technique

A 200 g portion of the sample is digested with 2000 ml of 1.25 per cent. sulphuric acid as in the castor process. After washing the undigested material by decantation it is transferred to a photographic dish and the undesirable seeds

are removed by picking them out. The weight of the dried seeds must be corrected for the small losses which occur as a result of the treatment.

DATURA STRAMONIUM

This can be determined by the Acid Fibre Technique, the dark blackish seeds being readily picked out and also any pieces of broken seeds if required. The Regulations are in fact only concerned with whole seeds but clearly the examination must be extended further when there are cases of possible poisoning.

The prepared husk is very characteristic, and it may be counted very easily by the lobelia technique.

LOLIUM TEMULENTUM

These are poisonous seeds which have been found occasionally in cereals and they are thought by some people to be the "tares" referred to in biblical times. The seeds are characterised by the very long "awn" which is present at the end of the grain. In the absence of this characteristic (i.e. when the awn is broken off) they are very difficult to distinguish from some grass seeds.

CROTALARIA

The Acid Fibre Technique can also be useful to show the presence of crotalaria, and also unhusked beech and bassia husk, and as far as the author knows it is the only way of detecting crotalaria. Crotalaria seeds have a very characteristic shape but the prepared husk is difficult to distinguish from husk of other legumes.

The Acid Fibre Technique will reveal some other weed seeds but they may or may not contain alkaloids, glucosides or other toxic substances. The prescribed limit of 3000 mg per kilo appears generous but it could well be too generous for some of the toxic seeds which very occasionally crop up.

A survey of the literature shows that the toxicology of many weed seeds which have been labelled as poisonous is still very uncertain and the author has pointed out on several occasions that, if limits are to be introduced for some of these so-called undesirable substances, they should be based on sound toxicology.

References

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DETERMINATION OF SOME SPECIFIED VEGETABLE TOXINS

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Aflatoxin

Aflatoxin is the name given to a group of toxins produced by a strain of the mould *Aspergillus flavus* when it grows on a number of organic materials,

THIN LAYER CHROMATOGRAPHY

We have found that the use of commercially prepared plates is advantageous. They are much more resistant to abrasion, and more regular in coating thickness than those produced with a Camag spreader. Thus they give more consistent R_F values.

There was a difficulty in comparing intensities from spots produced by 10 μ l and 40 μ l of solution. The larger amounts tend to produce a more diffuse spot, for some reason at a slightly higher R_F value. The volumes spotted can be kept to a maximum of 15 μ l by modifying the Official method in using three strengths of standard solution. The quantities and standard solutions used in the Official method and in the Modified method are as follows.

Official Methods I and II

- 10, 15, 20, 30 and 40 μ l of standard aflatoxin B_1 solution (0.1 μ g/ml).
- 10 μ l of sample extract, and superimposed on it, 20 μ l of standard B_1 solution.
- 10 and 20 μ l of sample extract.

Modified Method

- A. 5, 10, 15 μ l of aflatoxin B_1 solution (0.1 μ g/ml),
10, 15 μ l of aflatoxin B_1 solution (0.2 μ g/ml),
10 μ l of aflatoxin B_1 solution (0.4 μ g/ml).
- B. 10 μ l of sample extract and, superimposed on it, 10 μ l of standard aflatoxin B_1 solution (0.2 μ g/ml).

If two determinations are to be made on the same plate, the group of standards is placed in the centre, with one sample on each side.

Comparing results obtained by the official procedure with the modification, the results obtained by comparing spots from approximately equal volumes were lower than those from large volume standard spots. It would seem that the eye compares intensities and does not allow for a larger surface area.

CONFIRMATION OF IDENTITY OF B_1

On every occasion when aflatoxin appears to have been found in groundnut products its identity has been confirmed both by the sulphuric acid spray and the two-way chromatography; substances simulating B_1 have been found on occasion in soya and linseed products. When carrying out the two-way check on these materials as given on page 63 of the Amendment Regulations three different strength standard spots were put in the area B (diagram, page 65). This assists in confirmation of the amount as well as the identity of any B_1 present.

Although the Amendment Regulations recommend the use of the Method I (page 60) for determination of aflatoxin in most feedstuffs (including linseed and soya products) Method II has been found (page 66) to be preferable for these substances. Interfering fluorescing substances are present on occasion in both linseed and soya, which are not completely separated from aflatoxin in Method I, although they are in Method II.

Gossypol

Gossypol is the major pigment of cottonseed. It is yellow and was once

considered as a textile dye. It is a polyphenol and an aldehyde; it is present in the seed in discrete pigment glands. The glands are immediately ruptured by contact with water but not by hydrocarbons, chlorinated hydrocarbons or triglycerides.

The acute toxicity of gossypol is relatively low, but when fed in small amounts to mono-gastric animals over a long period, it can cause illness and even death. It has been reported that the toxicity is reduced by feeding higher levels of protein, and in some cases, of iron in the diet. The toxicity also varies with different animals, rabbits and pigs being among the most sensitive.

Gossypol as it exists in the kernel is extracted entirely by aqueous acetone. Under normal conditions of oil extraction, some of the gossypol becomes "bound" and it is not acetone-soluble. Free gossypol is physiologically active; bound gossypol is not. There is about 0.5 to 1.5 per cent. of gossypol in the seed: the amount remaining in the residue after oil extraction depends on the method of oil removal. If an expeller process is used, the heat and moisture present cause rupturing of the pigment glands, with loss of gossypol into the oil. Thus expeller meals, or solvent extracted expeller meals, tend to contain less gossypol than those meals prepared by direct solvent extraction, although nowadays in the United States such meals may be treated with organic amines or by other means to reduce the content of free gossypol. This is not the whole story however. The part of the gossypol which becomes bound does so, certainly in part, by combination with the protein present, involving the free epsilon-amino groups of the lysine present. By stringent conditions of steaming and cooking the free gossypol can be reduced very considerably, but it is accompanied by almost complete destruction of the protein value.

In the F & FS (Amendment) Regulations¹, there are methods for determination of both free and total gossypol, which differ only in the methods of extraction. In fact, only free gossypol is limited in the Regulations, and there appears to be no proposal to control total gossypol.

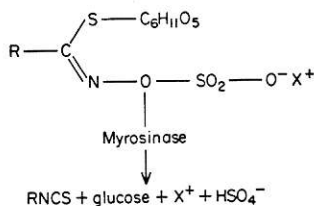
It has always been understood that free gossypol was toxic only to mono-gastric animals, and that ruminants were not affected by it. Certainly in the United States cattle have been fed on large amounts of cottonseed meal (up to 50 per cent.) in the diet without ill effect. Even whole raw cottonseed containing 1 per cent. of gossypol or more has been satisfactorily fed to cattle. However this may be, the EEC has decided to place a limit on the amount permissible, even in food for cattle.

The method in the Regulations is almost identical with the original of Pons & Hoffpaur², a method we have used occasionally, if not as a routine, over the past 15 years. The author cannot make any adverse criticism of it. It has always been found to be very easy to operate and it has given closely reproducible results.

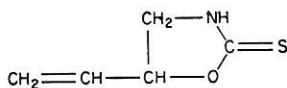
Vinyl Oxazolidone Thione (V.O.T.)

Seeds of rape and other cruciferous plants are processed commercially for the glyceride oils they contain, the residue having a high protein content and a reasonably well balanced amino-acid composition. Unfortunately, they contain large amounts of thioglucosides which on enzymatic hydrolysis yield both

volatile and non-volatile products. The major thioglucosides in rape (*B. napus*) and turnip rape (*B. campestris*) are gluco-napin and progoitrin, which have the generic formula:



The isothiocyanates produced include the volatile mustard oils, allyl, pentenyl and 3-butenyl isothiocyanates, which are estimated together and calculated as the allyl compound by the method given in the F & FS (Amendment) Regulations. However, if the product of hydrolysis should contain an OH group as in 2-hydroxy 3-butenyl isothiocyanate, cyclisation may occur with the formation of a non-volatile oxazolidone thione, which has the formula:



Vinyl oxazolidone thione is considered to be goitrogenic, and it has been stated that rape seeds, from *B. napus*, can produce up to 1.5 per cent. V.O.T., although there is so much work being effected on rape seed hybrids that no quoted figure can be realistic.

A method was put forward for determination of V.O.T. by L. R. Walter³ and modified by Applequist and Joseffson⁴. Basically, it consists in reacting the defatted meal with a myrosinase preparation, filtering and extracting with *iso*-octane to remove the "isothiocyanates", and then with diethyl ether to extract the V.O.T. The absorbance of the ether solution is then read at 230, 248 and 266 nm:

$$\begin{aligned}
 E_{248} - \frac{E_{230} + E_{266}}{2} &= E_{248} \text{ (corrected),} \\
 E_{248} \text{ (corrected)} \times 8.2 &= \mu\text{g V.O.T.}
 \end{aligned}$$

Unfortunately, although this appears at first sight to be a possible method, we have been unable to obtain consistent results and, failing news from the Government Laboratory which is at present examining the problem, there would appear to be no satisfactory method of estimation.

References

1. "The Fertilisers & Feeding Stuffs (Amendment) Regulations". S.I. 1976, No. 840, H.M.S.O., London, 1976.
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4. Applequist, L. A., and Joseffson, E., *J. Sci. Food Agric.*, 1967, **18**, 510.