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Undesirable Substances and Additives in Animal Feeding Stuffs

N. T. Crosby

Department of Industry, Laboratory of The Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ

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The theme for this session is "The Developing Role of the Enforcement Analyst". In the area of animal feeding stuffs, developments nowadays are primarily conditioned by activities of the European Community (EC) Commission. Following accession of the U.K. to the European Community in 1973, our legislation has been dominated by the harmonisation programme in which food and agriculture commodities have been increasingly subjected to common laws of quality, labelling, sampling and analysis. For animal feeding stuffs, there are now seven Council Directives concerned with composition, marketing, sampling and analysis. Many amendments to the original Directives have been promulgated, e.g. there are now 42 amendments to the Additives Directive alone. In addition to Council Directives, the Commission of the EC has published a number of Directives dealing specifically with the sampling and analysis of feeding stuffs. New procedures are continually under discussion in this area in an attempt to improve existing methods of analysis as new techniques became more widely available and accepted for general laboratory use. This review concentrates on the more recent changes in the legislation, particularly as it affects methods of analysis, and discusses some of the problems which remain and developments currently under way which attempt to resolve such problems.

Legislation enacted in Brussels is published in the Official Journal of the EC and has to be incorporated into the national legislation of all Member States. In the U.K., four new Statutory Instruments have been issued in the last year in order to fulfil our obligations. The Agriculture Act 1970 Amendment Regulations 1982[1] came into operation on 16 July 1982. For the first time, foods for pet animals have been included in the definition of an animal feeding stuff. A pet animal is defined as one normally kept and nourished but not consumed by man. Secondly, The Feeding Stuffs Regulations 1982[2] implement a whole cocktail of EC Directives on the marketing of straight and compound feeds, as well as the control of additives and undesirable substances in feeds. These Regulations also specify the particulars which (and which alone) are *permitted* to be included in a statutory statement in addition to the *required* particulars. The

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Regulations also specify ingredients amongst the particulars for inclusion in a statutory statement and ensure that where one ingredient is so specified, all must be listed in descending order of proportion by weight. Finally under the Agriculture Act 1970, the Feeding Stuffs (Sampling and Analysis) Regulations 1982[3] came into operation on 25 February 1983 and principally affect the manner of taking samples. Hence, the old Fertiliser and Feeding Stuff Regulations 1973 and subsequent amendments have now been entirely superseded. Parallel legislation has also been issued under the Medicines Act 1968. These Regulations[4] came into operation on 3 March 1983 and revoke the Medicines (Animal Feeding Stuffs) (Enforcement) Regulations 1976.
The main changes introduced into the methods to be used for sampling are as follows.

(a) More incremental samples have to be taken and different quantities are specified for the aggregate and final samples.
(b) The new Regulations include special provisions for the sampling of materials containing undesirable substances likely to be distributed non-uniformly, e.g. aflatoxins.
(c) As pet foods are now covered by the Regulations for the first time, special provisions for the sampling of these products were required. For example, unopened cans can be used, if by the opening of same, the composition or analysis would be affected.

Analysis of Animal Feeding Stuffs

The analytical methods prescribed in the new regulations[3] are essentially the same as those described in the old regulations, except that the Lane and Eynon method for sugars has been included for use only until 25 February 1984, when the Luff-Schoorl method alone can be used. In connection with the Luff-Schoorl method, it is important to note that the preparation of the reagent as described in paragraph 3.8 is critical and the composition should be checked by titration against a standard solution of sugar.

As a consequence of the inclusion of pet foods within the feeding stuff legislation, the analytical methods developed over the years for use with feeds will now have to be applied to sample types for which they were not intended, since many pet foods are closer in composition to human foods than to animal feeds. Foods for dogs and cats can be classified into three separate categories depending on their moisture content.

1. Canned foods containing >80% moisture.
2. Intermediate moisture foods in the range 20–40% moisture.
3. Biscuit-type foods containing <15% moisture.

At the present time, certain of the methods are not applicable to pet foods, for example as follows.

1. The method for moisture is not applicable to pet foods containing more than 14 per cent moisture. The Statutory Method leads to surface crusting and up to 24 h may be required in order to satisfy the criterion that the weight loss should be less than 2 mg between successive weighings. Products containing

glycols as humectants will give erroneous results for moisture content on oven drying at 100°C.

2. The methods for the determination of the oil content of feeding stuffs cannot be applied to pet foods since hydrolysis with acid is required to release the fat prior to extraction with light petroleum. Discussions are proceeding in Brussels and the replacement of diethyl ether by light petroleum and a hydrolysis method have now been agreed, subject only to agreement on the final text of each method.[6]

The new Regulations[3] include the Carr-Price spectrophotometric method for Vitamin A (retinol) in feeds despite the fact that many laboratories are now using a method based on HPLC. The Analytical Methods Committee of the Royal Society of Chemistry is currently evaluating such a method using a number of feeding stuff substrates including pet foods. Preliminary findings suggest that the HPLC method is still subject to a fair degree of variation which is essentially independent of the level of vitamin A present in the substrate. However, the identification at lower levels and in the presence of substances which interfere in the Carr-Price procedure is likely to be more certain. The method may also be applicable to other fat-soluble vitamins, such as vitamin E.

Undesirable Substances

There is currently great interest within the EC in reducing levels of aflatoxin B_1 in animal feeding stuffs. In particular, it has been agreed that the level for B_1 in complementary feeds for dairy cattle should be reduced from 0·02 mg/kg to 0·01 mg/kg. As the current Statutory Method[3] has a lower limit of determination of 0·01 mg/kg, it is clear that a more sensitive method is required for enforcement purposes. Furthermore, the statutory method is not applicable to feeding stuffs containing citrus pulp. The application of HPLC to this problem has been described by a number of workers. The method of Howell and Taylor[5] uses Sep-pak cartridges at the clean-up stage and using HPLC can detect down to 0·001 mg/kg levels in a 50 g sample. An alternative procedure based on post-column derivatisation with iodine has also been described[6]. This method enhances the fluorescence by a factor of about 20 as shown in Figure 1. No interference from citrus pulp co-extractives has been detected (Figure 2).

Few statutory methods exist for other undesirable components of animal feeding stuffs. Rape seed oil is a valuable commodity for the manufacture of margarine and the residues can be employed in animal feeds, since rape seed protein possesses a well balanced distribution of essential amino acids being high in lysine and methionine. However, the use of rape seed meal in animal feeds is restricted by the presence of glucosinolates which produce goitrogens on hydrolysis. A GLC method for the determination of volatile mustard oil, expressed as total *iso*thiocyanates calculated as the allyl compound, has now been evaluated in Europe. The lower limit of determination of each *iso*thiocyanate is 20 mg/kg. Both GLC and HPLC methods are available for the determination of vinyloxazolidinethione (VOT) in animal feedings stuffs with a lower limit of determination of 200 mg/kg.

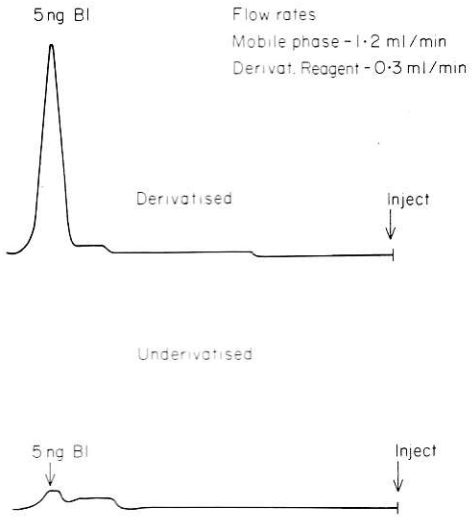

Fig. 1. Determination of aflatoxin B_1 by derivatisation with iodine.

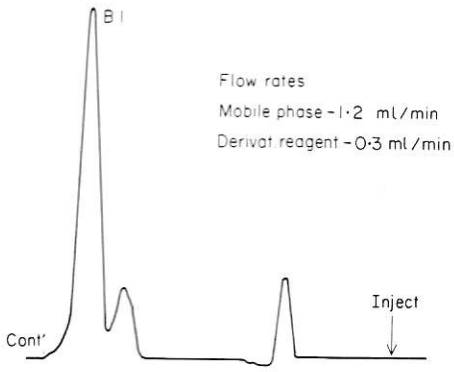

Fig. 2. Aflatoxin B_1 in feeds containing citrus pulp.

Medicinal Additives

Chemical methods of analysis for 12 coccidiostats are described in the Statutory Instrument[4]. These methods are spectrophotometric and, in many cases, are subject to interference from other drugs or from other co-extractives present in the feed. Methods based on HPLC are available for many of these compounds. Following collaborative trials, some procedures have been agreed and are awaiting agreement on the draft of the final text. Future years should see the replacement of many of these spectrophotometric methods by more rapid procedures based on HPLC. However, some analytical problems remain. In particular, the possible binding of medicinal additives to feed constituents needs to be investigated along with the stability of such additives to light and heat. The

latter parameter is especially important in view of current trade practice to market animal feeding stuffs in pelleted form using steam and binding agents which may well affect the quantity of active compound remaining in the pellet. Such studies are currently under way at the Laboratory of the Government Chemist along with a method for the qualitative identification of unknown medicinal compounds in animal feeding stuffs by means of HPLC.

In conclusion, the current areas of interest for analytical chemists involved in the enforcement of quality control of animal feeding stuffs are very wide and continually expanding. The traditional work involved in the analysis of meals and pellets remains, along with new problems of sampling and degradation of active compounds especially during pelleting. Furthermore, a new substrate, i.e. pet foods, has been added bringing its own special problems. The determination of ingredients poses particular difficulties which should ensure plenty of work for us all in the coming years.

References

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