



National Reference Laboratory for Feed Additives

End of Year Report
2022 - 2023

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Prepared by:

A handwritten signature in blue ink, appearing to read 'Kirstin Gray'.

Kirstin Gray

Project Manager – Feed Additives NRL Activities

Approved by:

A handwritten signature in blue ink, appearing to read 'Paul Hancock'.

Paul Hancock

Head of the Office of the Government Chemist

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1. Introduction

Retained EU Regulation No. 1831/2003 on *additives for use in animal nutrition* describes 'feed additives' as substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the following functions:

- (a) favourably affect the characteristics of feed,
- (b) favourably affect the characteristics of animal products,
- (c) favourably affect the colour of ornamental fish and birds,
- (d) satisfy the nutritional needs of animals,
- (e) favourably affect the environmental consequences of animal production,
- (f) favourably affect animal production, performance or welfare, particularly by affecting the gastro-intestinal flora or digestibility of feedingstuffs, or
- (g) have a coccidiostatic or histomonostatic effect.

Feed additives should not:

- (a) have an adverse effect on animal health, human health or the environment,
- (b) be presented in a manner which may mislead the user,
- (c) harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of animal products.

Antibiotics, other than coccidiostats or histomonostats, are not authorised as feed additives.

Depending on their functions and properties feed additives are allocated to one or more of the categories listed in Article 6 of Regulation (EC) No 1831/2003. The categories are:

- (a) technological additives: any substance added to feed for a technological purpose;
- (b) sensory additives: any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals;
- (c) nutritional additives;



- (d) zootechnical additives: any additive used to affect favourably the performance of animals in good health or used to affect favourably the environment;
- (e) coccidiostats and histomonostats.

LGC currently holds the role of National Reference Laboratory (NRL) for feed additives for Great Britain. The tasks and objectives for the feed additive NRL are as follows:

Part 1: NRL Core function

Objective 1. Secretariat services

Objective 2. Advice and representation within the UK and internationally

Objective 3. Production of standard operating procedures, codes of practice and guidance documents

Objective 4. Compliance assessment via audits and ring trials

Objective 5. Co-ordination within the UK of international initiatives

Objective 6. Communication of results and data use

Part 2: Feed Additive Regulated Product Authorisation

Objective 1. Infrastructure development

Objective 2. Maintenance of infrastructure

Objective 3. Core authorisation activities

This report provides an update for the National Reference Laboratory role for Feed Additives for the year April 2022 to March 2023.

2. Quality and staff competence

The Office of the Government Chemist at LGC, which hosts the feed additives NRL role, has continued to maintain its quality systems to the requirements of ISO17025. Annual assessments carried out by UKAS, together with internal audits, help ensure that quality and accreditation is maintained. The annual UKAS assessment of LGC's Teddington site was carried out in May. Several accredited methods held



and managed by the Office of the Government Chemist team were audited together with a review and assessment of the overall quality management system. Eight minor findings were raised against the team, all of which have been actioned to the satisfaction of UKAS and closed.

Feed additives cover a very wide range of chemicals / materials / substances, and therefore experience and competency is needed in a wide range of techniques. To maintain the ongoing competency of staff, proficiency tests (PTs) are regularly participated in. It is generally acknowledged that the number of PTs directly relevant to the analysis of feed and feed additives is limited. As the techniques used to analyse feed and feed additives are predominantly the same as those used for the analysis of food, participation in food PTs is carried out in addition to feed.

Table 1 gives examples of the analytes analysed in food PTs during 2022 and the techniques used. A similar range of PTs will be participated in during 2023.

Techniques	Example analyses
HPLC-UV	Vitamins
HPLC-FL	Aflatoxins
ICP-OES / ICP-MS	Trace elements / nutritional elements
GC-FID	Congeners and fatty acids
LC-MSMS	Coccidiostats / chloramphenicol
ELISA	Meat species / allergens
Gravimetric	Moisture, fat
General wet chemistry	Sulphur dioxide, formaldehyde

Table 1: Examples of analytes and the relevant techniques for PTs participated in during 2022.

It should be noted that while the costs of directly relevant feed rounds are attributed to the NRL role, the majority are funded either directly by LGC or through the Government Chemist programme. This enables the widest range of analytes / matrices to be covered in the most cost-effective way to each programme.

PT rounds available in 2022 / 2023 were reviewed and appropriate rounds chosen to cover a relevant range of analytes and techniques. The feed PTs participated in, together with the z-scores obtained are presented in Table 2.

Matrix	Analyte	Z-score
Pig ration	Moisture	0.8
Pig ration	Ash	0.6
Pig ration	Total oil	0.3
Pig ration	Protein	0.3
Pig ration	Vitamin E	-0.2
Pig ration	Zinc	0.9
Poultry feed	Chloramphenicol	0.7
Animal feed (Cereal based)	Arsenic (total)	0.9
Animal feed (Cereal based)	Cadmium	0.5
Animal feed (Cereal based)	Mercury (total)	1.4
Animal feed (Cereal based)	Nickel	1.0

Table 2: FAPAS feed PT rounds participated in during 2022.

In addition to participants' results, PT reports can also highlight a possible lack of laboratories undertaking some feed analyses. For example, according to FAPAS report Food Chem. Report 10182, the sample for the nutritional components in pig ration round was sent to 46 participants and, of the 41 participants that submitted any results, only 8 reported a result for vitamin E. Figure 1 is the chart taken from the FAPAS report (Food Chem. Report 10182) which shows the z-scores obtained by all participants. Six of the 8 (75 %) results were satisfactory. LGC is laboratory 18.

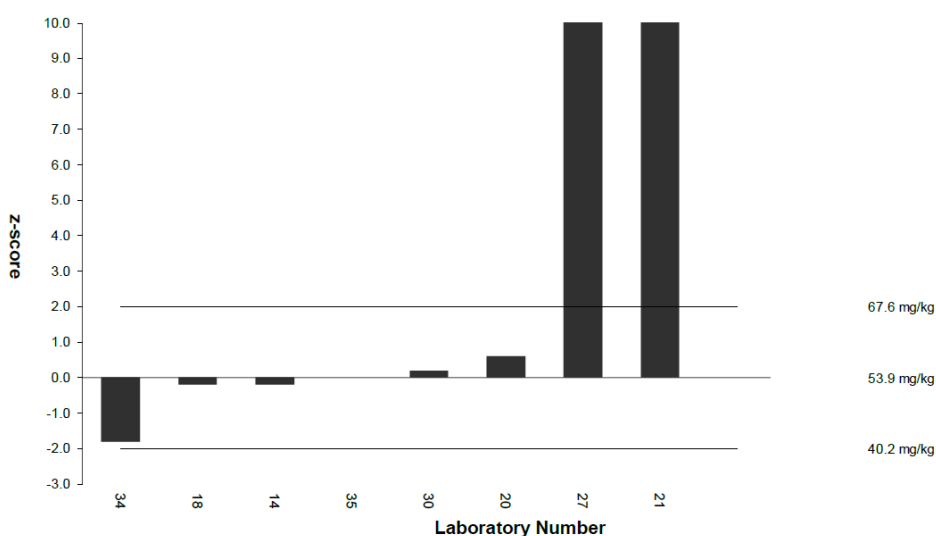


Figure 1: Vitamin E in pig ration – Z-scores for all participants (Chart taken directly from FAPAS (Fera Science Ltd) report Food Chem. Report 10182)



A PT round for chloramphenicol and coccidiostats in poultry feed sample was also participated in. A z-score of 0.7 was obtained for chloramphenicol, Figure 2 is a copy of the chart from FAPAS report Food Chem. Report 02484 showing the z-scores for all participants; LGC is lab number 28.

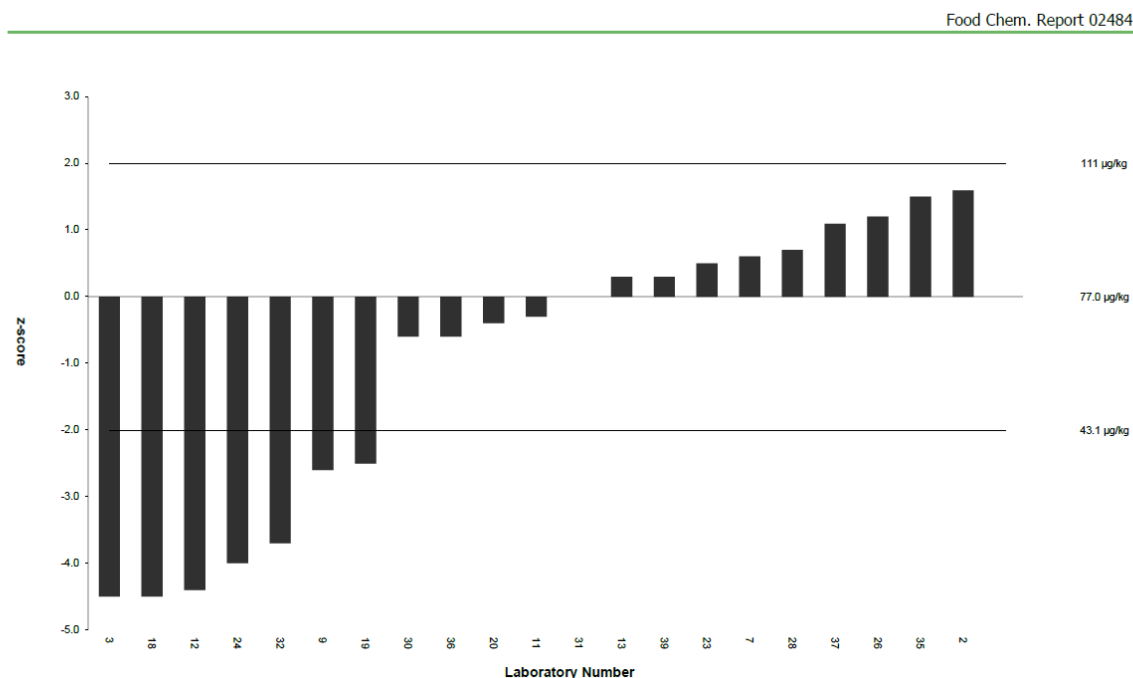


Figure 2: Chloramphenicol in poultry feed FAPAS PT – Z-scores for all participants (Chart taken directly from FAPAS (Fera Science Ltd) report Food Chem. Report 02484)

According to the report produced by FAPAS the median chloramphenicol result was chosen as the assigned value because of the low number results (21). From a review of the report, it appears that the results from the two labs that reported 0 µg/kg chloramphenicol (labs 3 and 18 in Figure 2) were not included when the median / assigned value of 77 µg/kg was calculated by FAPAS, but the results for labs 12 and 24 (2.19 and 9.6 µg/kg respectively) were. If the results of 2.19 and 9.6 µg/kg had also been excluded from calculation of the median, a z-score of 0.4 would have been obtained.

The FAPAS report states that chloramphenicol was spiked into the test material, however the spiking concentration was not provided.

In addition to chloramphenicol, the instructions received with the poultry feed sample listed 14 coccidiostats with a note stating that not all residues would be present. As it was not financially practical to purchase the standards and internal standards for all 14 coccidiostats solely for the analysis of one PT sample, the sample was analysed for 4 coccidiostats; narasin, monensin, DNC (for nicarbazin) and diclazuril. Of the 4 analysed, 3 were not present as the sample had been spiked only with diclazuril, lasalocid and robenidine, in addition to chloramphenicol. The FAPAS report stated that the median was chosen as the assigned value for robenidine because of the low number of results (23), the assigned value for diclazuril was given for information only because of the high associated uncertainty and for lasalocid it was not possible to set an assigned value due to the very high associated uncertainty. The graph from the FAPAS report showing the z-scores obtained by all participants for diclazuril is shown in Figure 3.

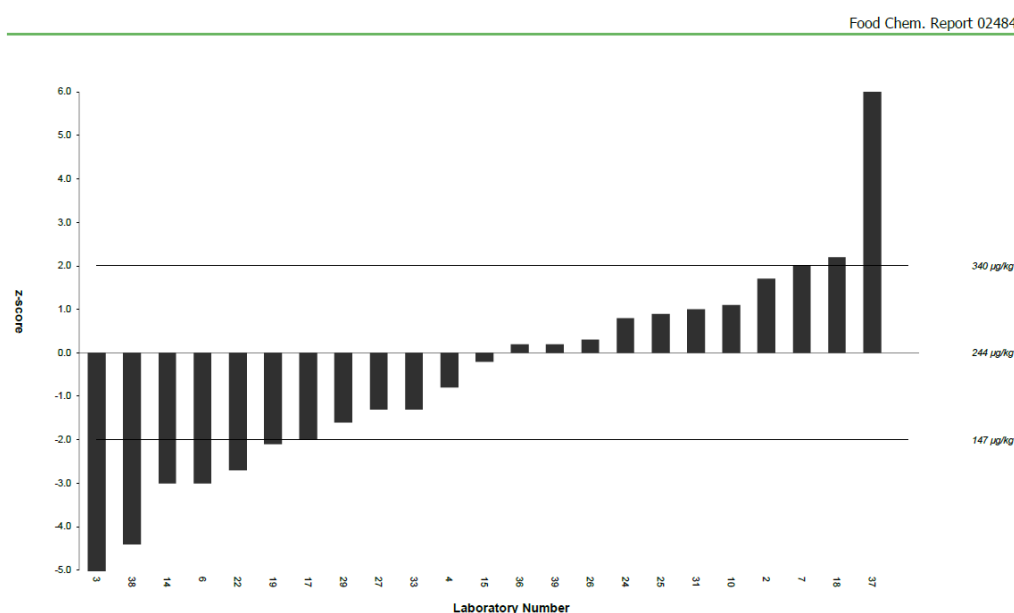


Figure 3: Diclazuril in poultry feed FAPAS PT – Z-scores for all participants (Chart taken directly from FAPAS (Fera Science Ltd) report Food Chem. Report 02484)

The result for diclazuril was not submitted due to poor linearity of the calibration line meaning a result for the sample could not be calculated with sufficient confidence. The method is being investigated, including a review of possible internal standards as labelled diclazuril is not available.



3.

4. Proficiency test review

A review of feed related proficiency tests was carried out. The only UK suppliers of feed related proficiency tests (PT) schemes are Fera Science Ltd (FAPAS) and LGC AXIO Proficiency Testing. A search was carried out on both companies' websites for feed PTs, the results are shown below.

Feed related PTs offered by Fera Science Ltd (FAPAS) in 2023

Product Code	Item Code	Start Date	Matrix	Analytes		
Proficiency Test						
Coccidiostats & Chloramphenicol in Poultry Feed Proficiency Test						
FCVD8-AFE15	02526	29/09/2023	Poultry Feed	coccidiostats & chloramphenicol (contaminant levels)	£306.00	+
Coccidiostats & Chloramphenicol in Poultry Feed Blank Proficiency Test						
BLVD30-AFE23	02526b	29/09/2023	Blank Poultry Feed	BLANK for coccidiostats & chloramphenicol (BLVD30)	£95.00	+
Veterinary Medicines in Animal Feed Proficiency Test						
FCVD17-AFE1	02533	29/11/2023	Animal Feed	Beta-agonists	£306.00	+
Veterinary Medicines in Animal Feed Blank						
BLVD17-AFE4	02533b	29/11/2023	Blank Animal Feed	BLANK for beta-agonists (BLVD17)	£95.00	+
Veterinary Medicines in Animal Feed Proficiency Test						
FCVD40-AFE1	02547	20/03/2024	Animal Feed	antibiotics	£306.00	+



Veterinary Medicines in Animal Feed Blank Material

BLPM1-AFE4	02547b	20/03/2024	Blank Animal Feed	BLANK (BLPM1)	£95.00	+
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Mycotoxins in Animal Feed (Cereal Based) Proficiency Test

FCMM5-AFE2	04461	02/12/2022	Animal Feed (Cereal Based)	contamination of matrix with two or more from the following list: aflatoxin B1, OTA, ZON, DON, FB1 & FB2 & total fumonisins (as a sum of FB1 & FB2)	£360.00	+
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Aflatoxins in Cereal Based Animal Feed Proficiency Test

FCMA2-AFE2	04471	06/04/2023	Animal Feed (Cereal Based)	aflatoxins B & G &/or total	£233.00	+
FCMA2-AFE2	04493	12/10/2023	Animal Feed (Cereal Based)	aflatoxins B & G &/or total	£233.00	+

Mycotoxins in Animal Feed (Cereal Based) Proficiency Test

FCMM5-AFE2	04498	30/11/2023	Animal Feed (Cereal Based)	contamination of matrix with two or more from the following list: aflatoxin B1, OTA, ZON, DON, FB1 & FB2 & total fumonisins (as a sum of FB1 & FB2)	£403.00	+
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Heavy Metals in Animal Feed (Cereal Based) Proficiency Test

FCCM46-AFE2	07462	24/11/2022	Animal Feed (Cereal Based)	selection of metals at natural/low levels	£212.00	+
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Heavy Metals in Animal Feed (Plant Origin) Proficiency Test

FCCM45-AFE23	07473	09/03/2023	Animal Feed (Plant Origin)	selection of metals at high levels	£212.00	+
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Heavy Metals in Animal Feed (Cereal Based) Proficiency Test

FCCM46-AFE2	07508	23/11/2023	Animal Feed (Cereal Based)	selection of metals at natural/low levels	£237.00	+
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Heavy Metals in Animal Feed (Plant Origin) Proficiency Test

FCCM45-AFE23	07521	07/03/2024	Animal Feed (Plant Origin)	selection of metals at high levels	£237.00	+
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Pesticide Residues (multi-residues) in Animal Feed (Cereal Based) Proficiency Test

FCPM2-AFE2	09163	11/10/2023	Animal Feed (Cereal Based)	pesticide residues (multi-residue)	£236.00	+
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Pesticide Residues (multi-residues) in Animal Feed (Cereal Based) Blank Proficiency Test

BLPM2-AFE21	09163b	11/10/2023	Blank Animal Feed (Cereal Based)	BLANK for pesticide residues (multi-residue) (BLPM2)	£75.00	+
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Nutritional Components in Poultry Ration Proficiency Test

FCNC21-AFE16	10185	07/12/2022	Poultry Ration	moisture, ash, total oil, protein, crude fibre, starch, total sugars, calcium & phosphorus	£387.00	+
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Nutritional Elements in Animal Feed Premix Proficiency Test

FCNE5-AFE17	10186	20/01/2023	Premix	calcium, magnesium, manganese, phosphorus, sodium & zinc	£214.00	+
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Nutritional Components in Dairy Ration Proficiency Test

FCNC20-AFE7	10187	16/03/2023	Dairy Ration	moisture, ash, total oil, protein, crude fibre, iron, magnesium, manganese & selenium	£387.00	+
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Nutritional Components in Pig Ration Proficiency Test

FCNC1-AFE14	10188	05/07/2023	Pig Ration	selection of nutritional components	£269.00	+
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Nutritional Components in Soybean Meal Proficiency Test

FCNC1-AFE20	10189	28/09/2023	Soybean meal	selection of nutritional components	£240.00	+
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Nutritional Components in Poultry Ration Proficiency Test

FCNC1-AFE16	10191	06/12/2023	Poultry Ration	selection of nutritional components	£433.00	+
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Nutritional Components in Premix Proficiency Test

FCNE1-AFE17	10192	25/01/2024	Premix	selection of nutritional elements	£240.00	+
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Nutritional Components in Dairy Ration Proficiency Test

FCNC1-AFE7	10193	01/03/2024	Dairy Ration	selection of nutritional components	£433.00	+
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Ochratoxin A in Animal Feed (Cereal Based) Proficiency Test

FCMO1-AFE2	17246	01/11/2023	Animal Feed (Cereal Based)	ochratoxin A	£233.00	+
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Mycotoxins in Animal Feed (Cereal Based) Proficiency Test

FCMF2-AFE2	22213	21/09/2023	Animal Feed (Cereal Based)	deoxynivalenol (DON), zearalenone (ZON), T-2 & HT-2 toxins & as a sum of T-2 & HT-2 toxins	£353.00	+
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Mycotoxins in Animal Feed Proficiency Test

FCMF11-AFE1	22218	19/01/2024	Animal Feed	Deoxynivalenol (DON), Deoxynivalenol 3-glucoside (DON-3-Glc) & 3Ac DON	£304.00	+
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Contaminants in Animal Feed (Cereal Based) Proficiency Test

FCCP5-AFE2	30138	29/06/2023	Animal Feed (Cereal Based)	melamine & cyanuric acid	£237.00	+
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Mycotoxins in Pet Dog Food Proficiency Test

FCMM18-PFO11	04481	06/07/2023	Pet Dog Food (Dry, Cereal Based)	☞ aflatoxin B1, DON, ZON, OTA, FB1 & FB2 & total fumonisins (as a sum of FB1 & FB2), T-2 & HT-2 toxins & as sum of T-2 & HT-2 toxins & 4,15-Diacetoxyscirpenol (DAS)	£564.00	+
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Heavy Metals in Pet Dog Food (Dried) Proficiency Test

FCCM46-PFO7	07482	18/05/2023	Pet Dog Food (Dry)	☞ selection of metals at natural/low levels	£237.00	+
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Nutritional Components in Pet Dog Food (Dry) Proficiency Test


FCNC1-PFO7	10190	09/11/2023	Pet Dog Food (Dry)	☞ selection of nutritional components	£269.00	+
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Feed related PTs offered by LGC AXIO Proficiency Testing in 2023

 6 - Salmonella (Detection) - Animal feed Product Code: PT-AF-06 PT Scheme: Animal Feed (AFPS) Brand: AXIO Matrix: Animal feed Analytes: Detection of Salmonella species 0 - 1,000 - Order sample →	 14 - Nutritional analysis of silage Product Code: PT-AF-14 PT Scheme: Animal Feed (AFPS) Brand: AXIO Matrix: Silage Analytes: Crude ash All % + 5 more Order sample →	 13 - Nutritional analysis - Fish Meal Product Code: PT-AF-13 PT Scheme: Animal Feed (AFPS) Brand: AXIO Matrix: Fish meal Analytes: Ash All % + 6 more Order sample →
 1 - Nutritional analysis - An... Product Code: PT-AF-01 PT Scheme: Animal Feed (AFPS) Brand: AXIO Matrix: Animal feed Analytes: Acid detergent fibre All % + 11 more Order sample →	 9 - Nutritional analysis - Wet Pet Food Product Code: PT-AF-09 PT Scheme: Animal Feed (AFPS) Brand: AXIO Matrix: Animal feed (Wet Pet Food) Analytes: Ash insoluble in hydrochloric acid All % + 8 more Order sample →	 11 - Listeria monocytogenes/s... Product Code: PT-AF-11 PT Scheme: Animal Feed (AFPS) Brand: AXIO Matrix: Animal Feed Analytes: Detection of Listeria monocytogenes 0 - 1,000 cfu/25g + 1 more Order sample →




<p>5 - Mycotoxins in Animal feed</p> <p>Product Code: PT-AF-05 PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal feed Analytes: Aflatoxin B1 All µg/kg + 5 more</p> <p>Order sample →</p>	<p>7 - Indicator organisms (Enum...</p> <p>Product Code: PT-AF-07 PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal feed Analytes: Enumeration of Enterobacteriaceae 0 - 100,000 cfu/g + 4 more</p> <p>Order sample →</p>	<p>2 - Minerals and trace elemen...</p> <p>Product Code: PT-AF-02 PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal feed Analytes: Arsenic All mg/kg + 16 more</p> <p>Order sample →</p>
<p>8 - Minerals and trace elemen...</p> <p>Product Code: PT-AF-08 PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal feed (Premix) Analytes: Arsenic All mg/kg + 16 more</p> <p>Order sample →</p>	<p>10 - Clostridium perfringens/...</p> <p>Product Code: PT-AF-10 PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal Feed Analytes: Enumeration of Clostridium perfringens 0 - 100,000 cfu/g + 3 more</p> <p>Order sample →</p>	<p>15 - Microbiological Contaminants</p> <p>Product Code: PT-AF-15 PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal Feed Analytes: Bacillus cereus (enumeration) 0 - 100,000 cfu/g + 3 more</p> <p>Order sample →</p>
<p>06KB - Salmonella presence/ab...</p> <p>Product Code: PT-AF-06KB PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Dried petfood/kibble</p> <p>Order sample →</p>	<p>07KB - Microbiological qualit...</p> <p>Product Code: PT-AF-07KB PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Dried petfood/kibble</p> <p>Order sample →</p>	<p>07AF - Microbiological qualit...</p> <p>Product Code: PT-AF-07AF PT Scheme: <i>Animal Feed (AFPS)</i> Brand: AXIO Matrix: Animal feed</p> <p>Order sample →</p>



06AF - Salmonella presence/ab...

Product Code: PT-AF-06AF
PT Scheme: Animal Feed (AFPS)
Brand: AXIO
Matrix: Animal feed


[Order sample →](#)



19 - Fusarium mycotoxins in oats

Product Code: PT-AF-19
PT Scheme: Animal Feed (AFPS)
Brand: AXIO
Matrix: Oats/oat flour


[Order sample →](#)



20 - Fusarium mycotoxins in maize oil

Product Code: PT-AF-20
PT Scheme: Animal Feed (AFPS)
Brand: AXIO
Matrix: Maize oil

[Order sample →](#)



18 - Anti-oxidants in copra oil

Product Code: PT-AF-18
PT Scheme: Animal Feed (AFPS)
Brand: AXIO
Matrix: Copra oil

[Order sample →](#)

To summarise, the results of the searches showed that in 2023 FAPAS will be running the following 20 feed related PTs (Table 3).

Analytes	Number of rounds
Nutritional components	6
Mycotoxins	6
Heavy metals	3
Pesticides	1
Nutritional elements	1
Contaminants	1
Beta-agonists	1
Coccidiostats and chloramphenicol	1

Table 3: Summary of FAPAS feed PTs for 2023



Feed additives that are included in the various nutritional component rounds are:

- Vitamin E
- Zinc
- Iron
- Manganese
- Selenium.

LGC AXIO have the following 19 feed PTs scheduled for 2023 (Table 4). It should be noted, however, that some of the LGC AXIO PTs described above are run more than once a year.

Analytes	Number of rounds
Microbiology	9
Nutritional analysis	4
Mycotoxins	3
Minerals and trace elements	2
Antioxidants	1

Table 4: Summary of LGC AXIO feed PTs for 2023

The nutritional analysis rounds do not contain any feed additive analytes, however some of the analytes in the minerals and trace elements rounds, for example copper, zinc, selenium and iron, are feed additives.

A search of the European PT Information System (EPTIS, www.eptis.bam.de) for proficiency tests related to feed and feed additives came back with rounds from the following companies worldwide.

- Serviço Nacional de Aprendizagem Industrial SENAI, Brazil
- LACM/División de Metrología, Chile
- Analysis Capability Assessment System of Chinese Academy of Inspection and Quarantine, China
- Central Institute for Supervising and Testing in Agriculture (UKZUZ), Department of Proficiency Testing Programmes, Czech Republic
- BIPEA Proficiency Testing, France
- SCHEMA (Scheme for Chemical Measurement Assessment), General Chemical State Laboratory, Greece



- Test Veritas S.r.l., Italy
- Kenya Bureau of Standards, KEBS, Kenya
- Mol Labs Ltda, Mexico
- Trilogy Europe B.V., Netherlands
- MasterLab, Netherlands
- Associacio Catalana de Fabricants D'Aliments Compostos, ASFAC, Spain
- Center for laboratory proficiency testing, Department of Science Service, Thailand
- Metrology service Ltd., Ukraine
- Fera Science Ltd, UK
- LGC AXIO Proficiency Testing, UK
- AAFCO Proficiency Testing Program, United States

Although PT rounds from all of the above companies were listed when a search for 'feed' was carried out on the EPTIS database, a large number of the entries had not been updated for over at least 2 years, and many of the companies did not have current rounds.

The companies with the apparent largest offering of PTs were reviewed:

- Trilogy Europe B.V., Netherlands – No feed related PTs are currently being offered.
- BIPEA, France - Each year BIPEA organizes annual proficiency testing programs, available from July to June of the following year. Each program consists of a number of rounds involving one or more samples and a laboratory that enrolls in a program will receive all related samples. The current feed (raw materials and finished products) program consists of 21 samples, with approximately 2 samples each month. Trace elements and vitamins A, D3 and E are listed as analytes, however the analytes vary according to the nature of the sample, therefore it is assumed that not every sample will contain every analyte.
- Metrology Service Ltd, Ukraine – One animal feedingstuffs PT is run each year but the analytes are related to the quality of the feed, for example moisture, fat, ash and fibre, rather than feed additives.



In conclusion, there appears to be no more appropriate current commercial PTs available for feed analytes than those being offered by FAPAS and LGC AXIO Proficiency Testing. It is also preferable to use UK based suppliers for PTs where possible as it eliminates potential delays in deliveries from overseas and the associated issues with Customs.

5. Horizon scanning

The Rapid Alert System for Food and Feed (RASFF) is reviewed monthly to try and detect trends and help identify possible future issues. The product categories reviewed are: feed additives, feed materials, feed premixtures, compound feed and pet food, together with the risk category of feed additives.

In the twelve months from April 2022 to March 2023 there were 236 RASFF notifications for the above categories. Together, salmonella and Enterobacteriaceae accounted for 50 % (119) of the notifications. The next highest categories were unauthorised additives with 17 notifications, mycotoxins (aflatoxins and T2 and HT2) with 14 notifications and the presence of ragweed (*Ambrosia spp.*) with 10 notifications. The number of notifications in 2022 - 2023 was similar to the number in 2021 – 2023; 217 notifications in total, 92 for salmonella and Enterobacteriaceae, 16 for ragweed and 15 unauthorised additives. Table 5 gives the reasons for all 236 notifications from April 2022 – March 2023.

	Number of RASFF notifications April 2022 - March 2023
Salmonella	104
Unauthorised additives	17
Enterobacteriaceae	15
Mycotoxins (Aflatoxins and T2 + HT2)	14
Ambrosia / ragweed	10
Pesticides (Chlorpyrifos, bifenthrin, cypermethrin, glyphosphate, paraquat)	9
Lead	9
Dioxins	7
Foreign bodies / glass / plastic / metal	6
Mould	6
Arsenic	4
Product of animal origin / ruminant DNA	4
Ergot	3
Not stated	3
Selenium	3
Cadmium	2
Copper	2
Fluorine	2
GMO	2
Asbestos	1
Cyanide	1
Datura seeds	1
Dodder seeds	1
Diclazuril	1
Insufficient checks	1
Ivermectin	1
Mercury	1
Microbiological	1
Nitrites	1
Salinomycin	1
Urea	1
Vitamin D3	1
Zinc	1
Total	236

Table 5: RASFF notifications involving feed additives, feed materials, feed premixtures, compound feed and pet food in the 12 months from April 2022 to March 2023



During the 12 months reviewed there were 17 RASFF notifications relating to the presence of unauthorised feed additives. The unauthorised additive notifications were:

- Non-compliance with paragraph 4 of Annex II to Regulation (EC) 767/2009 regarding the content of Vitamin D3 in complementary feed.
- Non-authorised additive boric acid in liquid complementary feedingstuff from the Netherlands.
- Unauthorised feed additive (sodium acetate) in dietetic complementary feed for calves.
- Non-authorised additives: E100 and E150a in feed for dogs.
- Unauthorised vitamin D2 in complete dry vegan puppy food from the United Kingdom offered online for sale.
- Unauthorised substance chlorpropham in organic corn flakes (feed) from Austria.
- Unauthorised substance ethoxyquin in supplementary feed for dairy cows from Germany, produced in Italy with raw material from the United States.
- Unauthorised substance ethylene oxide in xanthan gum from China.
- Ethylene oxide (sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide) in the feed additive - choline chloride.
- Ethylene oxide in red pepper powder from Germany.
- 2-chloroethanol in wheat protein from France.
- 2-chloroethanol in wheat gluten for animal feed from France.
- Unauthorised feed additive inositol in bird feed supplement from Czech Republic.
- Non-approved feed additive cannabidiol in CBD oil for pets from Czech Republic.
- Non-approved feed additive cannabidiol in pet food from the Netherlands.
- Non-approved feed additive CBD in CBD oil for pets from Poland.
- Non-approved feed additive CBD and L-theanine in CBD chews for pets from USA.
- Non-approved feed additive CBD in CBD oil for pets from Poland.



There were five RASFF notifications relating to CBD in various products, but as noted previously, whilst the notifications were recorded as unauthorised feed additives on the RASFF system, in Great Britain the Veterinary Medicines Directorate (VMD) consider that 'veterinary products containing cannabidiol (CBD) are veterinary medicines and should be regulated as such'.

No significant trends were identified from the RASFF notifications.

6. Standardisation activities

Kirstin Gray is a member of CEN/TC 327/WG3 "Feed additives and drugs".

A hybrid meeting of CEN/TC 327/WG 3, Feed additives and drugs was held at the premises of Biosafe in Kuopio, Finland and via Zoom on the 14th October 2022. The meeting primarily focussed on DNA-based methods for strain identification from animal feeds and PFGE typing of *Lactobacilli*, *Pediococci*, *Enterococci* and *Bacilli* in animal feeds. Paul Hancock and Kirstin Gray both attended via Zoom.

Microorganisms used as feed additives are authorised within the EU under their specific strain identification number. To enforce the specific conditions of use there is a need for suitable analytical methods that allow identification of these microorganism at strain level. The DNA fingerprinting method pulsed field gel electrophoresis (PFGE) was validated, and a CEN standard drafted with the aim of it being applied within the frame of official control. During the development of the document, it became clear that there was a preference from some parties for the method not to be part of the official method cascade with the associated obligation to use the PFGE method for official control. Therefore, it was decided to issue the PFGE protocol as a CEN *technical specification* rather than as CEN *standard*. This modification would allow official control laboratories to alternatively apply widely available DNA sequencing methods if preferred.

During the meeting, the technical aspects of both methods were discussed. The aim of the meeting was to have a dedicated discussion within a broader group of European experts in the field, mainly from EU Member States' authorities and relevant industry representatives, on a 'future proof' way forward for the



identification of microorganisms used as feed additives within the EU. The aim, as an outcome of the meeting and possible future meetings, would be to investigate whether a corresponding CEN work item could be launched to draft and publish suitable guidance documents regarding DNA sequencing methods.

The main conclusions of the meeting were:

- The identification of microorganisms used as feed additives at strain level is a targeted analysis as the objective of the analysis is to check whether the strain of a microorganism present in a feed additive under investigation is identical to the specific strain number indicated on the label or supporting documents. Corresponding information from the feed additive dossier and/or the authentic feed additive sample are required to carry out the interpretation of the results of analysis.
- The PFGE method is a well-established DNA fingerprinting method which has been recently validated via an interlaboratory study. However, the method is not used by many laboratories anymore.
- Recently laboratories more often use DNA sequencing methods such as whole genome sequencing (WGS) for various purposes. These methods would in principle be capable to identify microorganisms at strain level.
- Alignment with the work of CEN/TC 463 "Microbiology of the food chain" is envisaged, especially regarding EN ISO 23418:2022 Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance. The purpose would be to check whether such guidelines would be applicable to the scope of TC 327/WG 3.
- Some industry representatives are reluctant to share information from WGS with other laboratories. As an alternative, industry may identify specific nucleotides that would allow for identification at strain level by means of DNA sequencing methods and which could be shared with other laboratories.

A follow-up online meeting was held Tuesday 7th February 2023; Kirstin Gray attended.



7. Meetings

Meetings have been held quarterly with the FSA to discuss activities carried out under the NRL role and any issues that have arisen.

A meeting was held with the FSA on 23rd May 2022 to discuss the reported issues with the determination of vitamin A in samples of feed.

Paul Hancock and Selvarani Elahi attended the FSA's NRL symposium on 5th October 2022.

A catch-up meeting was held with the FSA on 8th December; vitamins analyses, and a review of Retained EU Regulation 152/2009 were discussed.

A catch-up meeting with the FSA, including members of the Feed Delivery Team, was held on 30th January. The main topic of discussion was current and future sampling programmes.

On 28th February 2023 Paul Hancock attended a Feed Protein Innovation webinar where one of the topics discussed was the development of alternative proteins for use in animal feed.

8. NRL network meeting

The annual network meeting was held, in conjunction with the NRL for GMO in food and feed, via Teams on the morning of Wednesday 8th March 2023 and was an opportunity for the NRLs to provide relevant updates on methods, enforcement, training and other relevant activities, as well as providing a forum for exchange of information and for participants to raise any further training needs and support requirements. The FSA and FSS also attended and provided a brief update on relevant scientific and policy areas. A copy of the agenda from the meeting is provided in Annex 1, the Feed Additives NRL presentation is in Annex 2.

The meeting was attended by 16 participants from 8 UK OLs plus the Isle of Man, 10 participants from FSA, 2 from FSS and 6 from LGC.

During the meeting the OLs were asked the following four questions:

- Number of samples analysed for feed additives in last 12 months?
- Which analytes?
- Has there been any issues with the methods used?
- Are there any methods for other additives that you require support with?



The responses were as follows:

OL 1

- They do not get many feed additives, only 1 for heavy metals.
- They do analyse additives in compound feed.
- Issues – Vitamin A

OL2

- Analysed 14 feed samples for vitamins A and E in the last year.
- Participated in 1 feed PT.
- Issues – None in particular. If there were issues it would likely be with vitamin E.

OL3

- Number of samples analysed for feed additives in the last 12 months? 0
- Which analytes? Not applicable due to previous answer of zero
- Has there been any issues with the methods used? Not applicable due to previous answer of zero
- Are there any methods for other additives that you require support with? Difficult to anticipate due to no requirement to use methods.

OL4

- They receive a fairly high number of feed samples, both from Port Health and Trading Standards. In excess of 500 feed samples since 01/01/22 but many of these were bird nuts for aflatoxins and feed materials/additives for heavy metals and nutrition testing (oil/ash/fibre/protein).
- They carry out the agriculture work for two counties.
- 16 samples analysed for vitamins, 1 for copper and 1 for urea.
- Issues – Urea by UV can give variable results due to matrix.
- A more straightforward method for vitamin D and B vitamins would be useful.
- Methods for additives where there are maximum permitted levels as there can be over supplementation in products such as dietetic foods.
- Heat can be an issue when preparing (milling) samples.



OL5

- Around 90 feed samples analysed, mainly for nutritional elements.
- Issues – Vitamin A

OL6

- 61 feed samples analysed for trace metals and vitamins A and E.
- Issues – Homogeneity can be a problem, results improve when a larger sample size is used.
- Would be interested in the SPE method for vit A.

OL7

- 56 agriculture samples last year of which 54 were feeding stuff and 2 were feed materials.
- The feed materials were tested for heavy metals only.
- Of the 54 feeding stuffs all were checked for compliance against the statutory statement and nutritional metals where appropriate included.
- 7 samples were also tested for lysine and methionine levels.
- No problems with methodology have been reported.

OL8

- No animal feed samples analysed in the last year.
- Areas where help needed – PCR (GMO) and mycotoxins.

OL9

- Around 110 feed samples analysed in the last year.
- 25 samples analysed for lysine and methionine
- A few samples analysed for metals.
- Other analytes included: vitamin C, vitamin B1, vitamins A and E, vitamin B6 and coccidiostats. (The methods used for each of these analytes were not stated.)
- Issues – none currently.



Comments on retained EU Regulation 152/2009

Samples are currently screened using an alternative method, for example Dumas for nitrogen rather than Kjeldahl. Could options for screening be included in the Regulation?

Some methods are old fashioned. Could performance criteria be used rather than described methods? It is useful to have described methods with performance criteria as a starting point, but include the option to use an alternative method with the same or better performance?

General comments

There is a deficiency in PTs, would organising a PT for vitamins A and E in feed be a possibility for the NRL?

Is there a need to look at speciated metals in feed? (For information, it is understood that Fera Science Ltd, the GB National Reference Laboratory for heavy metals and nitrogenous compounds, is looking at metal speciation).

Methods for unauthorised additives? There are problems using food methods as they do not always work for feed, for example formaldehyde and ethoxyquin. Port Health have asked for 'screens' for unauthorised additives.

A very small number of samples are currently being taken throughout Great Britain. West Yorkshire Joint Services used to analyse around 100 feed samples a year and they were not located in an agricultural area of the country.

9. OL Advice, training and support

Following a request from one of the OLs the method for the determination of vitamin A has been investigated due to reported issues with the procedure including poor recoveries and variation in replicate results. The methods for the determination of vitamin A is described in Annex IV, Methods of analysis to control the level of authorised additives in feed, of Retained EU Regulation 152/2009. The scope of the method is stated as 'This method makes it possible to determine the level of vitamin A (retinol) in feed and premixtures. Vitamin A includes all-trans-retinyl alcohol and its cis-isomers which are determined by this method. The content of vitamin A is



expressed in International Units (IU) per kg. The limit of quantification is 2 000 IU vitamin A / kg'. The principle is as follows: the sample is hydrolysed with ethanolic potassium hydroxide solution and the vitamin A is extracted into light petroleum. The solvent is removed by evaporation and the residue is dissolved in methanol. The content of vitamin A is determined by reversed phase high performance liquid chromatography (RP-HPLC) using a UV or a fluorescence detector.

Following conversations with an OL regarding the reported problems with the determination of vitamin A in feed, 6 samples were provided by the OL. The samples, all complementary, compound feed, were some of those where problems had been encountered and were said to contain in the region of 8,000 to 10,000 IU/kg vitamin A. Trials of the method described in Retained EU Regulation 152/2009 were carried out beginning with a comparison of standards and detectors (UV and fluorescence) and confirmation of linear range and LOD.

An aliquot of retinyl acetate solution was taken through the extraction procedure described in EURL 152/2009 and a range of calibration standards prepared from the resulting extract. These standard solutions were compared to those prepared directly from retinol. As can be seen from the two graphs in Figure 4 the two sets of calibration standards compared well.

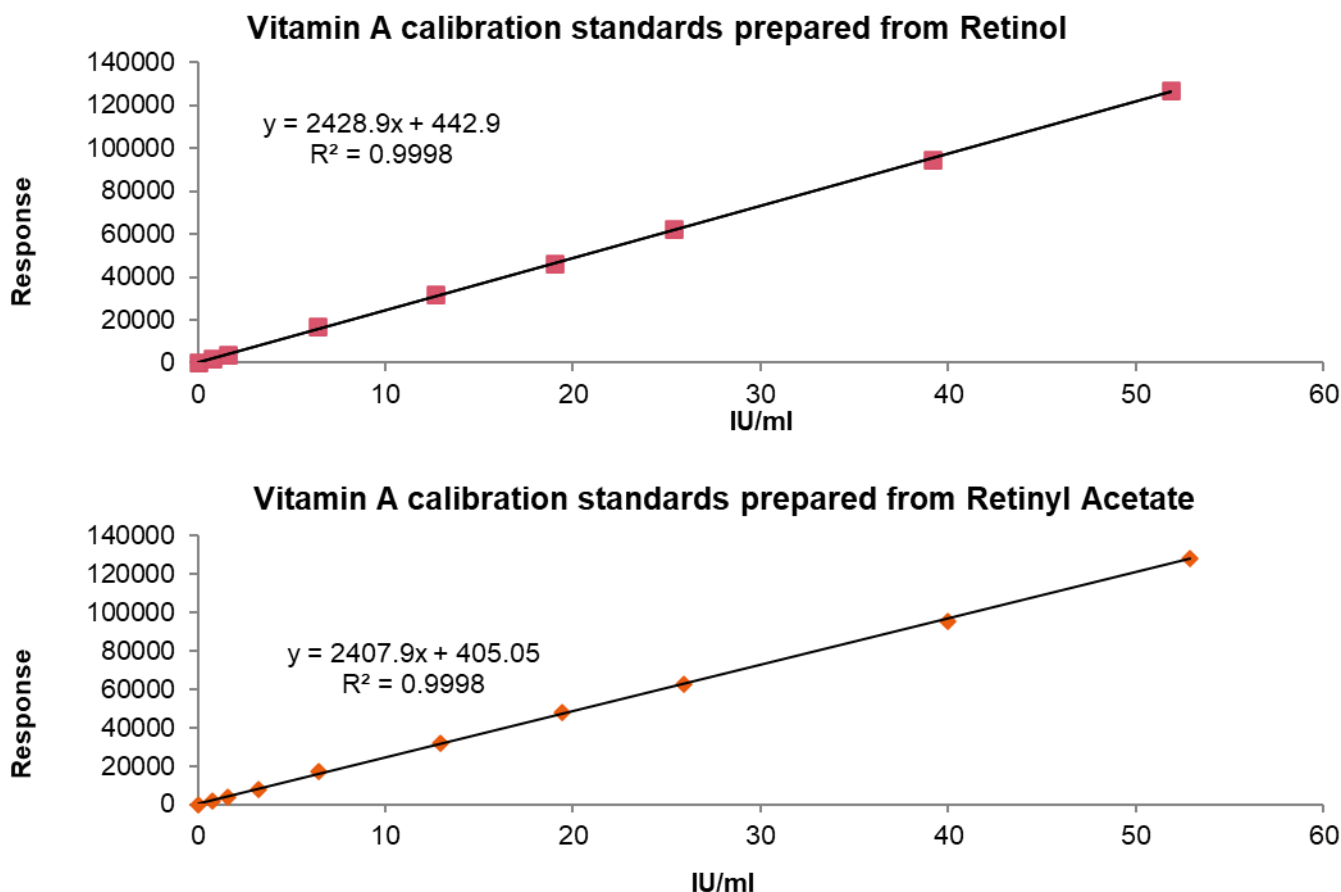


Figure 4: Comparison of calibration standards prepared from retinyl acetate and retinol.

The calibration range in the method is 2.8 to 28.0 IU/ml, this was extended to approximately 1.0 – 50 IU/ml, and the calibration was proved to be linear over this range for standards prepared using both retinyl acetate and retinol.

Multiple replicates of the samples were analysed to assess the repeatability. As an example, Table 6 shows the results obtained for sample 136073. The relative difference between the results (the difference between the highest and lowest as a percentage of the highest) was determined as 18 % when UV detection was used, and 19 % when fluorescence detection was used. Retained EU Regulation 152/2009 requires that the repeatability, as the difference between the results of two parallel determinations carried out on the same sample, must not exceed 15 % relative to the higher result. The variation in vitamin A concentration for sample 136073 was greater than 15 % but only minimally.

Sample	UV detection (IU/kg)	Fluorescence detection (IU/kg)
136073 A	7763	7301
136073 B	6422	6230
136073 C	7305	7018
136073 D	6348	5910
136073 E	6560	6131
Mean	6880	6518
Relative difference	18 %	19 %

Table 6: Vitamin A results obtained for sample 136073.

Table 7 shows the vitamin A results from multiple extractions from a different sample. As can be seen the variation between results is significantly higher indicating that the issue with poor repeatability and therefore variable recoveries is more likely due to issues with the sample. To try and identify if the poor repeatability was due to sample inhomogeneity a portion of the sample was further ground in a coffee mill and re-analysed, however the repeatability was no better.

Sample	Sample as received - UV detection (IU/kg)	Sample ground before analysis - UV detection (IU/kg)
1360175 A	909	23,008
1360175 B	2,956	3,887
1360175 C	6,411	18,036
1360175 D	6,125	7,012
1360175 E	11,460	1,873
1360175 F	1,266	7,102
Mean	4,854	10,153
Relative difference	92 %	92 %

Table 7: Vitamin A results obtained for sample 136073.



Investigations into the methods are ongoing.

10. Official Laboratory capability

The Government Chemist has worked with the Food Standards Agency to conduct an annual survey to assess UK OL capability and capacity to undertake the analysis of food and feed to support official controls. The survey was sent to OLs with a deadline for responses of 19th August 2022. Key findings were provided to the FSA for the purposes of including some information in a paper to the FSA board for its September meeting. A final report is being drafted and will be shared with FSA, Defra & FSS.

11. Reporting

Monthly activity logs and quarterly reports have been completed and the annual report describing activities carried out in the last year submitted and a copy placed on the NRL webpage.

12. Legislation updates

Under the Government Chemist programme regular reviews of legislation relating to feed and food are carried out. With regards to EU legislation, the following feed additives authorisations and renewals were published between April and September 2022:

The authorisation of:

- Butylated hydroxyanisole as a feed additive for cats,
- Bitter orange extract as a feed additive for certain animal species,
- A preparation of the leaves extract of *Melissa officinalis* L. as a feed additive for all animal species,
- A preparation of lemon extract as a feed additive for all animal species,
- Great mullein tincture as a feed additive for certain animal species,
- A preparation of 3-nitrooxypropanol as a feed additive for dairy cows and cows for reproduction
- A preparation of *Lactiplantibacillus plantarum* DSM 26571 as a silage additive for all animal species.

- Expressed lemon essential oil, residual fraction of expressed lemon oil distilled, distilled lemon essential oil (volatile fraction) and distilled lime essential oil as feed additives for certain animal species
- Essential oil from *Origanum vulgare ssp. hirtum letsw.* as a feed additive for certain animal species
- Vitamin B12 in the form of cyanocobalamin produced by *Ensifer adhaerens* CNCM I-5541 as a feed additive for all animal species
- Ethyl acrylate, pentyl isovalerate, butyl 2-methyl butyrate, 2-methylundecanal, (2E)-methylcrotonic acid, ethyl (E,Z)-deca-2,4-dienoate, butan-2-one, cyclohexyl acetate, 3,4-dimethylcyclopentan-1,2-dione, 5-ethyl-3-hydroxy-4-methylfuran-2(5H)-one, phenethyl butyrate, hexyl phenylacetate, 4-methylacetophenone, 4-methoxyacetophenone, 3-methylphenol, 3,4-dimethylphenol, 1-methoxy-4-methylbenzene, trimethyloxazole and 4,5-dihydrothiophen-3(2H)-one as feed additives for all animal species
- Allura Red AC as a feed additive for small non-food producing mammals and ornamental birds
- Monosodium glutamate produced by fermentation with *Corynebacterium glutamicum* KCCM 80187 as a feed additive for all animal species
- Potassium diformate as a feed additive for weaned piglets, pigs for fattening and sows, and repealing Implementing Regulation (EU) No 333/2012
- Olibanum extract from *Boswellia serrata Roxb.ex Colebr.* as a feed additive for horses and dogs
- A preparation of *Propionibacterium freudenreichii* DSM 33189 and *Lentilactobacillus buchneri* DSM 12856 as a feed additive for all animal species
- Ylang ylang essential oil from *Cananga odorata* (Lam) Hook f. & Thomson as a feed additive for all animal species
- A preparation of *Lactobacillus acidophilus* CECT 4529 as a feed additive for all poultry species and categories, other than laying hens and chickens for fattening, and for ornamental birds and amending Implementing Regulation (EU) 2017/2275
- Buchu leaf essential oil from *Agathosma betulina* (P.J. Bergius) Pillans as a feed additive for all animal species.

- Expressed orange essential oil, distilled orange essential oil and folded orange oils from *Citrus sinensis* (L.) Osbeck as feed additives for all animal species.
- L-glutamic acid and monosodium glutamate produced by *Corynebacterium glutamicum* NITE BP-01681 as feed additives for all animal species.
- 6-phytase produced by *Komagataella pastoris* (DSM 23036) as a feed additive for all avian species and for all pigs and repealing Implementing Regulation (EU) No 98/2012
- 3-ethylcyclopentan-1,2-dione, 4-hydroxy-2,5-dimethylfuran-3(2H)-one, 4,5-dihydro-2-methylfuran-3(2H)-one, eugenol, 1-methoxy-4-(prop-1(trans)-enyl)benzene, α -pentylcinnamaldehyde, α -hexylcinnamaldehyde and 2-acetylpyridine as feed additives for certain animal species.
- Camphor white essential oil from *Cinnamomum camphora* (L.) J. Presl. and cinnamon tincture from *Cinnamomum verum* J. Presl. as feed additives for all animal species.
- L-lysine sulphate produced by *Escherichia coli* CGMCC 7.398 as a feed additive for all animal species.
- Manganese lysinate sulphate as a feed additive for all animal species
- Endo-1,4-beta-xylanase produced by *Trichoderma longibrachiatum* CBS 139997 and alpha-galactosidase produced by *Aspergillus tubingensis* ATCC SD 6740 as a feed additive for chickens for fattening, chickens reared for laying, minor poultry species for fattening and reared for laying, and ornamental birds
- Lanthanum carbonate octahydrate as a feed additive for cats
- L-valine produced by *Escherichia coli* CCTCC M2020321 as a feed additive for all animal species.
- L-methionine produced by *Corynebacterium glutamicum* KCCM 80245 and *Escherichia coli* KCCM 80246 as feed additives for all animal species.
- L-lysine monohydrochloride and L-lysine sulphate produced by fermentation with *Corynebacterium glutamicum* CGMCC 14498 as feed additives for all animal species.

The renewal of the authorisation of:

- a preparation of *Bacillus velezensis* DSM 15544 as a feed additive for weaned piglets and the authorisation for all avian species and categories and
- the renewal of the authorisation of sodium benzoate as feed additive for weaned piglets, the new authorisation for weaned piglets of other Suidae.

Amendment of authorisation:

- Amending Implementing Regulation (EU) 2018/1039 as regards the terms of authorisation of copper (II) chelate of amino acids hydrate as a feed additive for all animal species.
- Amending Implementing Regulation (EU) 2017/1490 as regards the terms of authorisation of manganese chelate of amino acids hydrate as a feed additive for all animal species.
- Amending Implementing Regulation (EU) 2016/1095 as regards the terms of authorisation of zinc chelate of amino acids hydrate as a feed additive for all animal species.
- Amending Implementing Regulation (EU) 2017/2330 as regards the terms of authorisation of iron (II) chelate of amino acids hydrate as a feed additive for all animal species.
- Amending Implementing Regulation (EU) 2019/804 as regards the terms of authorisation of the organic form of selenium produced by *Saccharomyces cerevisiae* CNCM I-3060 as feed additive for all animal species.

The denial of authorisation of:

- Ethoxyquin as a feed additive belonging to the functional group of antioxidants and repealing Implementing Regulation (EU) 2017/962.

With regards to ethoxyquin, Regulation (EU) 2017/962 has been repealed by Commission Implementing Regulation (EU) 2022/1375 of 5 August 2022 concerning the denial of authorisation of ethoxyquin as a feed additive belonging to the functional group of antioxidants. The authorisation of the additive ethoxyquin was suspended by Commission Implementing Regulation (EU) 2017/962 as EFSA could not conclude on the efficacy and safety of ethoxyquin for animals, consumers and the environment, due to an overall lack of data submitted by the applicant. In

particular, no conclusion was possible on the absence of genotoxicity of the metabolite ethoxyquin quinone imine and concerns were raised as to the possible mutagenicity of the impurity p-phenetidine. Supplementary data was supplied by the applicant, but it was EFSA's opinion that they 'could not conclude on the safety of the additive ethoxyquin at any level for long-living and reproductive animals, considering that the additive contains p-phenetidine, a recognised possible mutagen which remains as an impurity in the additive'. Therefore, it was assessed that the authorisation of ethoxyquin as a feed additive belonging to the functional group 'antioxidants' should be denied.

The Feed Additives (Authorisations) Regulations 2022 make provision as regards the authorisation of feed additives under Regulation (EC) 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

[The Food and Feed \(Miscellaneous Amendments\) Regulations 2022](#) amend various provisions following EU exit, particularly by removing cross references to EU legislation and inserting various tables into domestic statutory instruments.

Amendments include legislation concerning food contact materials, food additives, genetically modified organisms and animal feeding stuffs. Similar legislation applies in the devolved administrations: [The Food and Feed \(Miscellaneous Amendments\) \(Wales\) \(EU Exit\) Regulations 2022](#) and [The Food and Feed \(Miscellaneous Amendments\) \(Scotland\) Regulations 2022](#).

[The Feed Additives \(Authorisations\) \(Wales\) Regulations 2022](#), [The Feed Additives \(Authorisations\) \(Scotland\) Regulations 2022](#) and [The Feed Additives \(Authorisations\) \(England\) Regulations 2022](#) authorise the following new and renewals of feed additives in Wales and England respectively.

- Authorisation of a preparation of Manganese chelate of lysine and glutamic acid (identification number 3b509) as feed additive for all animal species.
- Authorisation of a preparation of *Lactobacillus buchneri* (DSM 29026) (identification number 1k20759) as a feed additive for all animal species.
- Authorisation of a preparation of Serine protease (EC 3.4.21.-) produced by *Bacillus licheniformis* (DSM 19670) (identification number 4a13) as a feed additive for chickens for fattening.

- Authorisation of a preparation of *Bacillus licheniformis* (DSM 28710) (identification number 4b1828) as a feed additive for laying hens, minor poultry species for laying, poultry species for breeding and ornamental birds.
- Authorisation of a preparation of 6-phytase (EC 3.1.3.26) (identification number 4a32) as a feed additive for all poultry species, ornamental birds, piglets, pigs for fattening, sows, minor porcine species for fattening or reproduction.
- Authorisation of Decoquinat (Deccox[®]) (identification number 51756i, formerly E756) as a feed additive for chickens for fattening.
- Authorisation of Decoquinat (Avi-Deccox[®] 60G) (identification number 51756ii) as a feed additive for chickens for fattening.
- Renewal of authorisation of Pyridoxine hydrochloride (vitamin B₆) (identification number 3a831) as a feed additive for all animal species.
- Renewal of authorisation of a preparation of *Saccharomyces cerevisiae* (CNCM I-4407) (formerly *Saccharomyces cerevisiae* NCYC Sc.47) (identification number 4b1702) as a feed additive for calves for rearing.
- Renewal of authorisation of a preparation of *Clostridium butyricum* (FERM BP-2789) (identification number 4b1830) as a feed additive for chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor avian species (excluding laying birds), weaned piglets and weaned minor porcine species, and its authorisation as a feed additive for chickens for fattening, suckling piglets and suckling minor porcine species.
- Renewal of authorisation (with modification) of a preparation of *Bacillus velezensis* (ATCC PTA-6737) (formerly *Bacillus subtilis* (ATCC PTA-6737)) (identification number 4b1823) as a feed additive for chickens for fattening, chickens reared for laying, ducks for fattening, quails, pheasants, partridges, guinea fowl, pigeons, geese for fattening and ostriches, and its authorisation as a feed additive extending existing uses to cover all minor poultry species (except for laying), ornamental birds, sporting birds and game birds.

13. EU legislation review

A copy of a presentation given by Frans Verstraete at the CEN/TC 327 plenary meeting was provided to CEN/TC 327 working group members. The presentation was titled 'Recent and future developments in legislation including status of future standardization request', and the main points of interest (noting some are outside of the scope of NRL activities, but are included for completeness) were:

Review of Regulation (EC) 152/2009

- The review is in the final stage with adoption and publication planned for early 2023.
- The following text is to be added, presumably in Annex II, General provisions on methods of analysis for feed, C Application of methods of analysis and expression of results, 3 Number of determinations, 'The aggregate sample is divided into 4 subsamples of equal weight of approximately 500 grams. One subsample is examined. In case the result of the subsamples is equal or below 50 % (analytical threshold) of the maximum level, the sample is compliant with the maximum level. If the result is above 50 % of the maximum level, another subsample needs to be examined and the average of the result of the 2 subsamples is used for checking compliance with the maximum level'.
- Determination of moisture (Annex III Methods of analysis to control the composition of feed materials and compound feed, A)
Repeatability (5.3) for pet food and dog chews to be increased from 0.2 % to 0.5 %.
- Determination of crude protein (Annex III Methods of analysis to control the composition of feed materials and compound feed, C)
Footnote to be added regarding the conversion factor from nitrogen to protein (presumably to be added in 6 Calculation of results).
- Determination of urea (Annex III Methods of analysis to control the composition of feed materials and compound feed, D)
A collaborative trial has shown that there is little difference in the performance criteria when urea is determined at 420 nm or 435. Therefore, the text in 7.3 that currently reads 'If the sample contains simple nitrogenous

compounds such as amino acids, the optical density shall be measured at 435 nm' to be replaced with 'The above results from collaborative trials do not indicate a significant difference in precision between urea measured at 420 nm or at 435 nm'.

- Determination of amino acids (Annex III Methods of analysis to control the composition of feed materials and compound feed, F)

The scope currently reads 'This method makes the determination possible of free (synthetic and natural) and total (peptide bound and free) amino acids in feed, using an amino acid analyser'. The text is to be changed to 'This method makes the determination possible of free (synthetic and natural) and total (peptide bound and free) amino acids in feed materials, compound feeds and premixtures containing less than 10%* of each amino acid, using an amino acid analyser.

*Note: This method has not been validated through a collaborative trial for premixtures containing more than 10% and feed additives. However, it is also applicable to these matrices with appropriate slight modifications provided that the method is then in-house validated. For additional information, consult <https://ec.europa.eu/jrc/en/eurl/feed-additives/authorisation>.'

- Determination of vitamin A (Annex IV Methods of analysis to control the level of authorised additives in feed, A)

Premixtures to be deleted from the scope. Also, clarification of the need to grind the sample prior to analysis (5.1) and the possibility of increasing the sample weight for samples with low vitamin A concentrations (5.2).

- Determination of vitamin E (Annex IV Methods of analysis to control the level of authorised additives in feed, B)

Premixtures to be deleted from the scope and observations (7.9).

- Determination of diclazuril (Annex IV Methods of analysis to control the level of authorised additives in feed, F)

The scope currently reads 'The method makes it possible to determine the level of diclazuril in feed and premixtures' but in principle it is also applicable to feed materials although they were not included in the collaborative trial. A foot note is to be added regarding the applicability to feed materials. Also, for

high fat content compound feed (>12 %) other HPLC based methods such as LC-MS can be used.

- Repeatability and reproducibility criteria is missing for certain methods of analysis.
- For all methods in the Annexes of 152/2009, relevant EN standards will be referenced, the aim being to make EN standards fully equivalent to the 'official methods' for control.
- Where there are no specific methods of analysis in the Annexes to 152/2009 there is a possibility that EN standards will be referenced. The EN standards that have been proposed are:
 - EN ISO 30024 – phytase activity
 - EN 17050: iodine by ICP-MS
 - EN 15784: Isolation and enumeration of presumptive *Bacillus* spp (other probiotics (EN 15785, 15786, 15787, 15788) and for yeast (EN15789)
 - EN 17212: melamine and cyanuric acid content by liquid chromatographic method with mass spectrometric detection (LCMS/MS)
 - EN 17053: trace elements, heavy metals and other elements in feed by ICP-MS (multi-method)
 - EN 16279: fluoride content after hydrochloric acid treatment by ion-sensitive electrode method (ISE)
 - EN 17194 Deoxynivalenol, Aflatoxin B1, Fumonisin B1 & B2, T-2 & HT-2 toxins, Zearalenone and Ochratoxin A in feed materials and compound feed by LC-MS/MS
 - EN 17270 theobromine in feed materials and compound feed, including cocoa derived ingredients, by liquid chromatography
 - EN 15510 calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-AES
 - EN 15621 calcium, sodium, phosphorus, magnesium, potassium,

sulphur, iron, zinc, copper, manganese and cobalt after pressure digestion by ICP-AES

- EN 17053 trace elements, heavy metals and other elements in feed by ICP-MS (multimethod)
- EN 17374 inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS
- EN ISO 6579-1: Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.
- EN 17362 pentachlorophenol (PCP) in feed materials and compound feed by LC MS/MS
- EN 17547 vitamin A, E and D content – Method using solid phase extraction clean-up and high-performance liquid chromatography.
- EN 17550 carotenoids in animal compound feed and premixtures by reverse phase high performance liquid chromatography – UV detection (RP-HPLC-UV)

Performance criteria approach for feed methods

The following questions were posed regarding method performance criteria:

- Are the analytical performance criteria under discussion (in replacement of the criteria established in Regulation (EC) 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs) for mycotoxins and plant toxins also applicable to feed?
- Are the analytical performance criteria provided for in Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs also applicable for feed?

Regulation (EC) 401/2006 Methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

The following are the anticipated replacement performance criteria for Regulation (EC) 401/2006:

- The average recovery should be between 70 and 120%. The criterion applies to all concentrations and all individual toxins. In exceptional cases, average recoveries outside the above range can be acceptable but shall lie within 50 to 130%, and only when the precision criteria for RSDr and RSDwR are met. Currently the limit for the acceptable recovery ranges is dependent on concentration and the toxin being determined, for example the acceptable recovery ranges for aflatoxins B1, B2, G1 and G2 are 50 to 120 % for concentrations <1.0 µg/kg, 70 to 110 % for 1 – 10 µg/kg and 80 to 110 % for >10 µg/kg, for T-2 and HT-2 toxins the acceptable recovery range is 60 to 130 % regardless of concentration.
- Precision shall be:
 - RSDr shall be ≤ 20%.
 - RSDwR shall be ≤ 20%.
 - RSDR should be ≤ 25%.

These criteria will apply to all concentrations. Where the maximum level applies to a sum of toxins, the criteria for precision will apply to both the sum and the individual toxins.

- Where a specific LOQ is not set, the LOQ shall be ≤ 0.5*ML, and preferably ≤0.2*ML. Where the maximum level applies to a sum of toxins, the LOQ of the individual toxins shall be ≤ 0.5*ML/n, where n = the number of toxins included in the ML definition.

It was stated that 'It is the view of the EURL mycotoxins & plant toxins in food and feed that the performance criteria for mycotoxins mentioned in the Regulation replacing 401/2006 (in finalisation) and plant toxins in food (Regulation in finalisation) are also fit-for-purpose and applicable to all feed matrices, including those matrices that do not have a food equivalent (such as "by-product" like citrus pulp, corn gluten, ... and compound feed"). It is recognised that some feed matrices are different from food commodities and analytically challenging, but the same is true within the food commodities (e.g. cereal products, spices, herbal preparations). Therefore, by default, the same performance criteria are proposed for feed. Only in exceptional toxin/matrix situations (not yet foreseen), deviations may be considered on a case-to-case basis'.



Regulation (EC) 333/2007 Methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

It was stated that the EURL for metals and nitrogenous compounds commented 'In general, the performance criteria for elements listed in Regulation (EC) 333/2007 are also applicable to all feed matrices. This includes both feed matrices similar to food as well as feed matrices that do not have a food equivalent. Only in special cases (element-matrix combinations) other criteria may be relevant to implement on a case-to-case basis (not yet foreseen).

With the current LOQ requirements listed in 333/2007 and the current feed MLs, the LOQ requirements for determination of trace elements in feed will in all cases be $1/5 \cdot ML$.

For many feed materials the MLs for elements are at a higher level compared to the MLs for trace elements in food matrices. Hence, the LOQ requirements for element determination in feed will be at a higher level compared to food. Therefore, methods used for food analysis will in most cases (if not all) comply with the LOQ requirement when used for feed analysis.

It should be noted that if the performance criteria in 333/2007 are implemented for feed analysis, then the LOQ requirements for compliance testing of feed will in several cases be much higher than is technically possible to achieve. Consequently, results from compliance testing may be reported as $< LOQ$ (with an LOQ at $1/5 \cdot ML$) and not provided as a numerical result, which could have been useful in e.g. relation to monitoring purposes'.

Directive 2002/32/EC on undesirable substances in animal feed

A possible new approach is under discussion for regulating mycotoxins in feed. Feed materials – Maintenance of the approach of establishing guidance levels based on the available occurrence data according to As Low As Reasonably Achievable (ALARA), and taking into account the year-to-year variation, climate change, the large variety of feed materials, etc...

Complete feed - The levels under discussion for complete feed are derived from reference points for adverse animal health effects (NOAEL /LOAEL), exceedance of which might consequently result in adverse animal health effect. Therefore, the

establishment of levels for complete feed as maximum levels (in the frame of Directive 2002/32/EC) is under consideration to ensure a high level of animal health protection.

Complementary feed - Application of Article 6 of Directive 2002/32/EC on undesirable substances: “Insofar as there are no special provisions for complementary feed, Member States shall prescribe that complementary feed may not, taking into account the proportion prescribed for their use in a daily ration, contain level of the undesirable substances listed in Annex I that exceed those fixed for complete feed.”

Consideration is being made to update the annexes to Directive 2002/32/EC on undesirable substances in animal feed to include the following:

- Lead in game meat for use in the production of pet food.
- Rye ergot in feed materials and compound feed containing unground cereals and date of application.
- delta-9-tetrahydrocannabinol (Δ 9-THC) in hemp seeds, hemp expeller, hemp seed oil, hemp flour, hemp fibre and complete feed.
- *Datura* sp.
- Dioxins and sum of dioxins and dioxin-like PCBs in fish oil, fish meal, hydrolysed fish protein and compound feed for fish.
- Arsenic in fish meal and corresponding footnote.
- Cadmium in copper (I) oxide.
- Nickel in certain feed materials.
- Endosulfan, heptachlor, hexachlorobenzene (HCB), hexachlorocyclohexane (HCH)-gamma isomers.
- p-phenetidine.
- Unavoidable cross-contamination of authorised feed additives into non-target feed.

Other topics under discussion include:

- Regulatory provisions on quinolizidine alkaloids in lupins and lupin derived feed materials.
- Ergot alkaloids.
- Nitrites and nitrates.

- Pyrrolizidine alkaloids.
- Hydroxymethylfurfural (HMF) in bee feed.
- Hydrocyanic acid (HCN) in linseed for birds.

Standardisation requests

The following are subjects for possible standardisation:

- Dioxins and dioxin-like PCBs
- Polycyclic aromatic hydrocarbons (PAHs)
- Urea
- Ethoxyquin in fish meal and fish feed
- p-Phenetidine in fish meal and fish feed
- Enumeration methods for probiotics
- Banned antibiotics
- Nickel, lanthanum and total chromium
- Speciation organic/inorganic selenium
- Nitrite and nitrate
- Extension of scope of multi-methods for mycotoxins
- Aflatoxin binders
- Tetrahydrocannabinol (THC)
- Alternaria toxins
- Determination of Sr-90 in feed
- Assess current methods for their RSDRs
- Characterisation of hydrolysed animal proteins
- Packaging materials in former food products

Please note that some of the above Regulations are not directly related to feed additives but are provided for information and completeness.

14. Review of retained EU regulation 152/2009

A review of the methods in Retained EU Regulation No 152/2009 laying down the methods of sampling and analysis for the official control of feed has been started.

The methods described in the Regulation are as follows:

ANNEX III

Methods of analysis to control the composition of feed materials and compound feed.

		Technique
A	Determination of moisture	Drying and weighing
B	Determination of moisture in animal and vegetable fats and oils	Drying and weighing
C	Determination of the content of crude protein	Digestion, distillation, titration
D	Determination of urea	Suspension in water, reaction with 4-DMAB, measurement of optical density
E	Determination of volatile nitrogenous bases	1. Extraction, microdiffusion, titration 2. Extraction, distillation, titration
F	Determination of amino acids (except tryptophan)	Extraction and ion-exchange chromatography with post-column derivatisation, photometric detection (amino acid analyser or HPLC system)
G	Determination of tryptophan	Hydrolysis, extraction, HPLC-FL
H	Determination of crude oils and fats	Acid digestion, solvent extraction, weighing
I	Determination of crude fibre	Acid / alkaline digestion, filtrations, ashing, weighing
J	Determination of sugar	Extraction, titration (Luff-Schoorl method)
K	Determination of lactose	Extraction, fermentation, titration (Luff-Schoorl method)
L	Determination of starch	Extraction, polarimetry
M	Determination of crude ash	Ashing, weighing
N	Determination of ash which is insoluble in hydrochloric acid	Acid digestion, ashing, weighing
O	Determination of carbonates	Dissolution, measurement
P	Determination of total phosphorus	Dry combustion or acid digestion, spectrophotometry
Q	Determination of chlorine from chlorides	Extraction, precipitation, titration

ANNEX IV

Methods of analysis to control the level of authorised additives in feed.

		Technique
A	Determination of vitamin A	Hydrolysis, liquid liquid extraction, HPLC
B	Determination of vitamin E	Hydrolysis, liquid liquid extraction, HPLC
C	Determination of the trace elements iron, copper, manganese and zinc	Dry ashing, atomic absorption spectrometry
D	Determination of halofuginone	Extraction, liquid liquid extraction, HPLC-UV
E	Determination of robenidine	Extraction, column clean-up, HPLC-UV
F	Determination of diclazuril	Extraction, SPE, HPLC-UV
G	Determination of lasalocid sodium	Extraction, HPLC-FL

ANNEX V

Methods of analysis to control undesirable substances in feed.

		Technique
A	Determination of free and total gossypol	Extraction, spectrophotometry
B	Determination of the levels of dioxins (PCDD/PCDF) and PCBs	Bioanalytical / GC-MS / GC-HRMS / GC-MS/MS

ANNEX VI

Methods of analysis for the determination of constituents of animal origin for the official control of feed.

		Technique
A	Light microscopy	Light microscopy
B	PCR	Polymerase chain reaction

ANNEX VII

Method of calculating the energy value of poultry feed.

		Technique
A	Calculation and expression of energy value	Calculation

ANNEX VIII

Methods of analysis to control illegal presence of no longer authorised additives in feed.

		Technique
A	Determination of methyl benzoate	Extraction, liquid liquid extraction, column clean-up, HPLC-UV
B	Determination of olaquinox	Extraction, HPLC-UV
C	Determination of amprolium	Extraction, HPLC-UV
D	Determination of carbadox	Extraction, HPLC-UV

An e-mail was sent to the OLs asking if they used the methods described in retained EU Regulation 152/2009. Of the 33 methods in the Regulations, the following 6 were said to be used by all 4 respondents, either frequently or occasionally:



ANNEX III Methods of analysis to control the composition of feed materials and compound feed:

- A. Determination of moisture
- C. Determination of the content of crude protein
- H. Determination of crude oils and fats
- I. Determination of crude fibre
- M. Determination of crude ash

ANNEX IV Methods of analysis to control the level of authorised additives in feed:

- C. Determination of the trace elements iron, copper, manganese and zinc

The following 15 methods were reported as not being used by any of the 4 responding OLs:

- Determination of amino acids (except tryptophan)
- Determination of tryptophan
- Determination of carbonates
- Determination of chlorine from chlorides
- Determination of halofuginone
- Determination of robenidine
- Determination of diclazuril
- Determination of lasalocid sodium
- Determination of free and total gossypol
- Determination of the levels of dioxins (PCDD/PCDF) and PCBs
- Light microscopy
- Determination of methyl benzoate
- Determination of olaquinox
- Determination of amprolium
- Determination of carbadox.

It was commented by one OL that for some analytes, as the Regulation methods are not specifically requested by customers, ISO methods are used instead. For example, ISO 5983-2:2009 or DUMAS (LECO) is used for the determination of



crude protein rather than the method described in Annex III C of retained EU regulation 152/2009 and microwave acid digestion followed by ICP-OES is used for the determination of trace elements rather than Annex IV C of retained EU regulation 152/2009.

15. Feed Additives NRL for NI

Contact was made with Austrian Agency for Health and Food Safety (AGES), the designated NRL for Feed Additives for NI. Contact details (nrl-ni-fsa@ages.at) were passed to the OLs at the network meeting.

16. NRL Forward Workplan

In 2023/24 the NRL core activities, e.g. horizon scanning, PT participation and dissemination of relevant information and advice as required, will continue. Input will also be made into the revision / review of any relevant Regulations, for example Retained EU Regulation 152/2009.

The systems and processes drafted for the evaluation of feed additive authorisation applicants and receipt and storage of reference samples will be maintained and dossiers will be evaluated as required.

17. NRL website

Information about LGC's NRL roles are found on our website at:

<https://www.lgcgroup.com/what-we-do/national-laboratory-and-government-roles/national-laboratory-roles/national-reference-laboratories/>. Additional webpages are to be added and will provide information on the authorisation process, guidance for submission of reference samples and standards relating to authorisation applications and a database of additives newly authorised for use in GB together with information on methods of analysis for control.

18. Feed Additive Authorisation

Feed additives are regulated products and as such require authorisation before use. Following Great Britain's exit from the EU, all new feed additives need to be authorised by the Competent Authority (FSA/FSS) before use in GB. Feed additives



that are currently approved for use in the EU can continue to be used in GB but an application is required to be submitted at least one-year prior to expiry to be progressed through the GB risk analysis process.

Under part 2 of the NRL contract, feed additive regulated product authorisation, the NRL is responsible for:

- the reception, preparation, storage and maintenance of reference samples and reference standards where applicable;
- evaluating the data provided by the applicant for authorisation to place the feed additive on the market, for the purpose of testing and evaluation or validation of the method for detection;
- submitting a full evaluation report to the FSA Risk team for each application within 3 months from the date of receipt of a valid application and payment of fee. This period can be extended for complex applications or where supplementary information is requested.

A shared mailbox has been set-up to facilitate communications between the NRL, the FSA and feed additive authorisation applicants. The e-mail address is feed.additives@LGCgroup.com.

A meeting was held with the FSA on 30th June 2022 to discuss the procedure for processing feed additive authorisation applications. Feedback on a draft guide on the authorisation process has since been supplied.

A proof-of-concept version of a compendium of authorisations which will appear on LGC's website, which was developed by an external web development contractor, has been tested and determined to be fit for purpose. This solution will now be soft-launched to the LGC live environment in order to enable a go-live as and when required.



Annex 1: OL network meeting agenda

Agenda

Meeting: Joint NRL (Feed Additives and GMOs) Network Meeting

Time & Date: 9:30 -13:30 Wednesday 8th March 2023

Venue: MS Teams Meeting

9.30 Welcome and introductions

- Apologies for absences
- Scope of the meeting

9.40 FSA update

10.00 Feed Additives National Reference Laboratory

- Updates and activities
- Future work programme
- Q&A

11.15 Break

11.30 GMO National Reference Laboratory

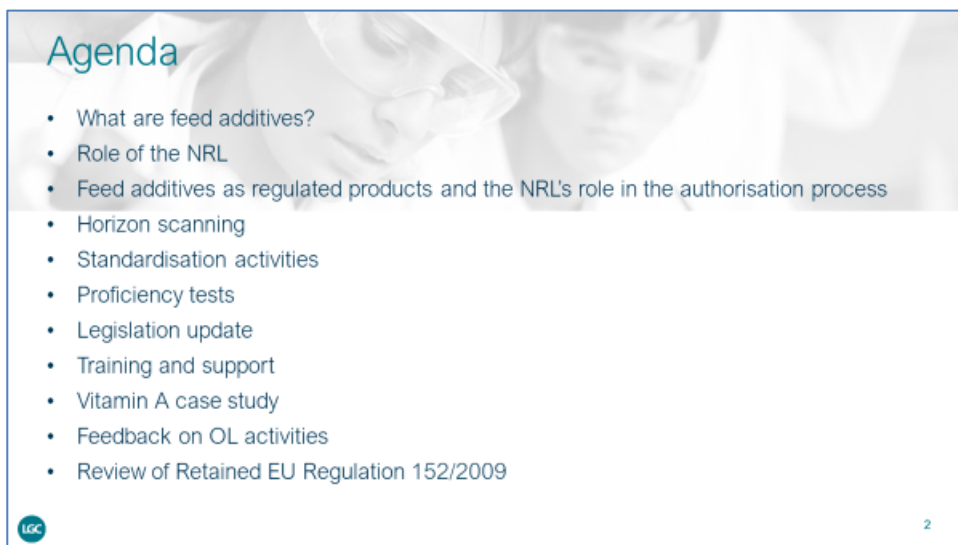
- Updates and activities
- Future work programme
- Q&A

12.45 Feedback / general discussion

13.00 Government Chemist Survey of Official Laboratories - Update

13:15 AOB
13.30 Close

Annex 2: Presentation from OL network meeting



Feed Additives

Retained EU Regulation (EC) No. 1831/2003 on additives for use in animal nutrition describes 'feed additives' as substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the following functions:

- (a) favourably affect the characteristics of feed,
- (b) favourably affect the characteristics of animal products,
- (c) favourably affect the colour of ornamental fish and birds,
- (d) satisfy the nutritional needs of animals,
- (e) favourably affect the environmental consequences of animal production,
- (f) favourably affect animal production, performance or welfare, particularly by affecting the gastrointestinal flora or digestibility of feedingstuffs, or
- (g) have a coccidiostatic or histomonostatic effect.



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Feed additives

Feed additives should not:

- (a) have an adverse effect on animal health, human health or the environment,
- (b) be presented in a manner which may mislead the user,
- (c) harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of animal products.



4

Feed additives

Depending on their functions and properties feed additives are allocated to one or more of the categories listed in Article 6 of Regulation (EC) No 1831/2003. The categories are:

- (a) technological additives: any substance added to feed for a technological purpose;
e.g. preservatives or gelling agents
- (b) sensory additives: any substance, the addition of which to feed improves or changes the organoleptic properties of the feed, or the visual characteristics of the food derived from animals;
e.g. colourants or flavourings
- (c) nutritional additives;
e.g. vitamins and trace elements
- (d) zootechnical additives: any additive used to affect favourably the performance of animals in good health or used to affect favourably the environment;
e.g. improving digestibility of feed
- (e) coccidiostats and histomonostats
to control gut parasites.



5

Role of the NRL

Retained EU Regulation 2017/625 on official controls and other official activities performed to ensure the application of food and feed law, describes the responsibilities and tasks of NRLs.

- ❖ NRL for feed additives for GB – LGC
- ❖ NRL for feed additives for NI - Austrian Agency for Health and Food Safety (AGES) (nrl-ni-fsa@ages.at)



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Role of the NRL - Part 1: Core function

- Dissemination of information between the Competent Authority and the Official Laboratories
- Advice and representation within the UK and internationally
- Production of standard operating procedures, codes of practice and guidance documents
- Compliance assessment via audits and ring trials
- Co-ordination within the UK of international initiatives



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Role of the NRL - Part 2: Feed Additive Regulated Product Authorisation

- Infrastructure development and maintenance
- Evaluation of applications



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Feed Additive Regulated Product Authorisation

Regulated Products

Certain food and feed products, called regulated products, require authorisation before they can be sold in the UK. For most regulated product types, once products or processes are authorised, they are listed in relevant legislation, which also sets out how they can be used.

As feed additives are regulated products, following Great Britain's exit from the EU all new feed additives need to be authorised by the FSA before they can be used in GB. Feed additives that are currently approved for use in the EU can continue to be used in GB but will need to go through the GB re-authorisation process when their current authorisation expires.

FSA guidance

Regulated products application guidance

What needs to be submitted as part of a regulated product application.

<https://www.food.gov.uk/business-guidance/regulated-products-application-guidance>

Feed additives authorisation guidance

Feed additive authorisation guidance and what needs to be submitted as part of a feed additive application.

<https://www.food.gov.uk/business-guidance/regulated-products/feed-additives-guidance>

Register of feed additives

The register of feed additives sets out a list of feed additives permitted for use in Great Britain and provides reference to the individual feed additive legislation.

The register does not replace retained EU Regulation 1831/2003 which is the legal basis for the placing on the market and use of individual feed additives.

<https://data.food.gov.uk/regulated-products>



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Role of the NRL with regards to feed additive authorisations

The main tasks of the NRL in relation to feed additive authorisations are as follows:

1. Scientific evaluation of the analytical methods submitted by applicants as regards their suitability for official control
2. Drafting and submitting evaluation reports to the FSA
3. Reception, storage and maintenance of the feed additive reference samples provided by the applicants.



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Scientific evaluation of methods of analysis proposed for official control

LGC will provide scientific method validation services to the Food Standards Agency as part of the authorisation process for placing on the market of feed additives for use in Great Britain.

The primary objective of the evaluation of the analytical methods is to establish whether the methods are suitable for enforcement of the conditions of use of the feed additives, for example whether the methods are capable of accurately determining the feed additive content at the set minimum and maximum concentrations.



14

Reference samples

Article 3 of Retained EU Regulation No 378/2005 on detailed rules for the implementation of Retained EU Regulation No 1831/2003 concerning applications for authorisations of feed additives states that any person submitting an application for an authorisation for a feed additive or for a new use of a feed additive shall supply three reference samples in a form in which the feed additive is intended to be placed on the market by the applicant.



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Reference standards

In addition, the applicant shall also provide reference standards of the pure active agents in the case of feed additives:

- belonging to the category zootechnical additives referred to in Article 6(1)(d) of Retained EU Regulation No 1831/2003, except feed additives consisting of or containing micro-organisms;
- belonging to the category coccidiostats and histomonostats referred to in Article 6(1)(e) of Retained EU Regulation No 1831/2003;
- falling within the scope of Community legislation relating to the marketing of products consisting of, containing or produced from genetically modified organisms (GMOs);
- for which Maximum Residue Limits have been established in Annex I or III of Retained EU Regulation No 2377/90 or following Retained EU Regulation No 1831/2003.



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Core NRL Role



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Horizon scanning

The Rapid Alert System for Food and Feed (RASFF) is reviewed monthly to try and detect trends and help identify possible future issues.

The product categories reviewed are:

- feed additives,
- feed materials,
- feed premixtures,
- compound feed and
- pet food.

The majority of notifications are not related to additives but are still of interest.



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Feed related RASFF notifications in 2022

In 2022 231 feed related RASFF notifications were recorded.

The top 10 reasons for notifications were:

Reason for recall	Total recalls in 2022
Salmonella	101
Unauthorised additive	15
Enterobacteriaceae	14
Arsenic / Copper / Manganese / Mercury / Lead / Selenium / Zinc	14
Mycotoxins (Aflatoxins, T2/HT2)	11
Ragweed (Ambrosia spp.)	8
Ruminant DNA / protein	8
Pesticide	7
Ethylene oxide	6
Metal particles / Metal / Plastic / Glass fragments	6



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RASFF – Unauthorised feed additives

Unauthorised additives	Number of recalls
CBD*	5
CBD and L-theanine	1
Astaxanthin	1
Boric acid	1
Cobalt carbonate, cobalt carbonate hydroxide monohydrate	1
E100 (curcumin), E150a (caramel)	1
Ethoxyquin	1
Inositol	1
Sodium acetate	1
Vitamin D2	1
Not stated	1

* CBD notifications were classified on RASFF as 'unauthorised additives', however it has been noted that in Great Britain VMD consider that 'veterinary products containing CBD are veterinary medicines and should be regulated as such'.




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Standardisation activities

Since September 2021 Kirstin Gray has been a member of CEN/TC 327/WG 3. Technical committee 327 - 'Animal feeding stuffs - Methods of sampling and analysis'


- CEN/TC 327/WG 1 Organic contaminants
- CEN/TC 327/WG 2 Composition
- CEN/TC 327/WG 3 Feed additives and drugs
- CEN/TC 327/WG 4 Elements and their chemical species
- CEN/TC 327/WG 5 Natural toxins
- CEN/TC 327/WG 6 Radioactivity measurements


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Standards published

The following standards were reviewed by CEN/TC 327/WG3 "Feed additives and drugs" and published.


- EN 15784: 2021 "Animal feeding stuffs: Methods of sampling and analysis — Detection and enumeration of *Bacillus spp.* used as feed additive"
- EN 15786: 2021 "Animal feeding stuffs: Methods of sampling and analysis — Detection and enumeration of *Pediococcus spp.* used as feed additive"
- EN 15787: 2021 "Animal feeding stuffs: Methods of sampling and analysis — Detection and enumeration of *Lactobacillus spp.* used as feed additive"
- EN 15788: 2021 "Animal feeding stuffs: Methods of sampling and analysis — Detection and enumeration of *Enterococcus (E. faecium) spp.* used as feed additive"
- EN 15789: 2021 "Animal feeding stuffs: Methods of sampling and analysis — Detection and enumeration of *Saccharomyces cerevisiae* used as feed additive"


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Standards published

The following 2 standards developed within CEN/TC 327/WG 3 were also published:

- EN 17547:2021 "Animal feeding stuffs: Methods of sampling and analysis - Determination of vitamin A, E and D content - Method using solid phase extraction (SPE) clean-up and high performance liquid chromatography (HPLC)"
- EN 17550:2021 "Animal feeding stuffs: Methods of sampling and analysis - Determination of carotenoids in animal compound feed and premixtures by high performance liquid chromatography - UV detection (HPLC-UV)"


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prEN 17697 'Animal feeding stuffs: Methods of analysis - PFGE typing of Lactobacilli, Pediococci, Enterococci and Bacilli in animal feeds'.

Comments were received that pulsed field gel electrophoresis (PFGE) is not suitable for the claimed scope of the standard and was no longer considered a routine procedure in microbiology labs.

The type of deliverable has been changed from an European Standard (EN) to a Technical Specification (TS).



Feed Proficiency Tests

A review of feed related proficiency tests was carried out. The only UK suppliers of feed related proficiency tests (PT) schemes are Fera Science Ltd (FAPAS) and LGC AXIO Proficiency Testing.

FAPAS feed PTs for 2023

Analytes	Number of rounds
Nutritional components	6
Mycotoxins	6
Heavy metals	3
Pesticides	1
Nutritional elements	1
Contaminants	1
Beta-agonists	1
Coccidiostats and chloramphenicol	1

LGC AXIO feed PTs for 2023

Analytes	Number of rounds
Microbiology	9
Nutritional analysis	4
Mycotoxins	3
Minerals and trace elements	2
Antioxidants	1

Note: Some of the LGC AXIO PTs described above are run more than once a year.

Legislation updates

Under the Government Chemist programme regular reviews of legislation relating to feed and food are carried out.

<https://www.gov.uk/government/organisations/government-chemist>

Updates include:

- Primary and secondary GB legislation (including devolved administrations)
- Updates to relevant EU legislation
- Additives approvals
- Other relevant information (e.g. Codex standards and FAO reports)



The Feed Additives (Authorisations) (Wales) Regulations 2022 and The Feed Additives (Authorisations) (England) Regulations 2022

- Authorisation of a preparation of Manganese chelate of lysine and glutamic acid (identification number 3b509) as feed additive for all animal species.
- Authorisation of a preparation of *Lactobacillus buchneri* (DSM 29026) (identification number 1k20759) as a feed additive for all animal species.
- Authorisation of a preparation of Serine protease (EC 3.4.21.-) produced by *Bacillus licheniformis* (DSM 19670) (identification number 4a13) as a feed additive for chickens for fattening.
- Authorisation of a preparation of *Bacillus licheniformis* (DSM 28710) (identification number 4b1828) as a feed additive for laying hens, minor poultry species for laying, poultry species for breeding and ornamental birds.
- Authorisation of a preparation of 6-phytase (EC 3.1.3.26) (identification number 4a32) as a feed additive for all poultry species, ornamental birds, piglets, pigs for fattening, sows, minor porcine species for fattening or reproduction.
- Authorisation of Decoquate (Deccox®) (identification number 51756i, formerly E756) as a feed additive for chickens for fattening.
- Authorisation of Decoquate (Avi-Deccox® 60G) (identification number 51756ii) as a feed additive for chickens for fattening.



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The Feed Additives (Authorisations) (Wales) Regulations 2022 and The Feed Additives (Authorisations) (England) Regulations 2022

- Renewal of authorisation of Pyridoxine hydrochloride (vitamin B6) (identification number 3a831) as a feed additive for all animal species.
- Renewal of authorisation of a preparation of *Saccharomyces cerevisiae* (CNCM I-4407) (formerly *Saccharomyces cerevisiae* NCYC Sc.47) (identification number 4b1702) as a feed additive for calves for rearing.
- Renewal of authorisation of a preparation of *Clostridium butyricum* (FERM BP-2789) (identification number 4b1830) as a feed additive for chickens reared for laying, turkeys for fattening, turkeys reared for breeding, minor avian species (excluding laying birds), weaned piglets and weaned minor porcine species, and its authorisation as a feed additive for chickens for fattening, suckling piglets and suckling minor porcine species.
- Renewal of authorisation (with modification) of a preparation of *Bacillus velezensis* (ATCC PTA-6737) (formerly *Bacillus subtilis* (ATCC PTA-6737)) (identification number 4b1823) as a feed additive for chickens for fattening, chickens reared for laying, ducks for fattening, quails, pheasants, partridges, guinea fowl, pigeons, geese for fattening and ostriches, and its authorisation as a feed additive extending existing uses to cover all minor poultry species (except for laying), ornamental birds, sporting birds and game birds.



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Training and support

As part of the role of the NRL we are here to support the labs where they have difficulties or issues with methods relating to feed additives.

An example of support is shown next:



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Determination of vitamin A

Retained EU Regulation 152/2009

Annex IV – Methods of analysis to control the level of authorised additives in feed (A)

Principle

The sample is hydrolysed with ethanolic potassium hydroxide solution and the vitamin A is extracted into light petroleum. The solvent is removed by evaporation and the residue is dissolved in methanol. The content of vitamin A is determined by reversed phase high performance liquid chromatography (RP-HPLC) using a UV or a fluorescence detector.

Scope

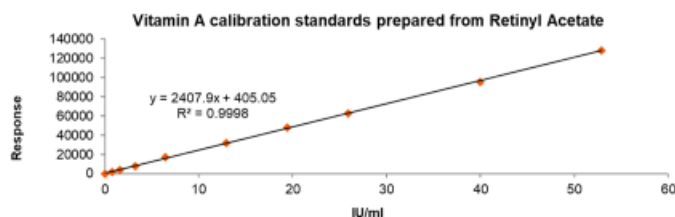
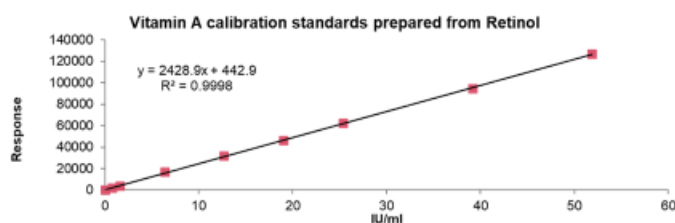
This method makes it possible to determine the level of vitamin A (retinol) in feed and premixtures. Vitamin A includes all-trans-retinyl alcohol and its cis-isomers which are determined by this method. The content of vitamin A is expressed in International Units (IU) per kg.

The limit of quantification is 2 000 IU vitamin A/kg.



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Determination of vitamin A - Calibration



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Determination of vitamin A - Repeatability

Sample	UV detection (IU/kg)	Fluorescence detection (IU/kg)
136073 A	7763	7301
136073 B	6422	6230
136073 C	7305	7018
136073 D	6348	5910
136073 E	6560	6131
Mean	6880	6518
Relative difference	18 %	19 %

Repeatability (Retained EU Regulation 152/2009)

The difference between the results of two parallel determinations carried out on the same sample must not exceed 15 % relative to the higher result.



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Determination of vitamin A - Repeatability

Sample	Sample as received - UV detection (IU/kg)	Sample ground before analysis - UV detection (IU/kg)
1360175 A	909	23,008
1360175 B	2,956	3,887
1360175 C	6,411	18,036
1360175 D	6,125	7,012
1360175 E	11,460	1,873
1360175 F	1,266	7,102
Mean	4,854	10,153
Relative difference	92 %	92 %



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Training and support

Are there any training needs?

For example:

- Discussions / training
- Guidance notes
- Proficiency tests.



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Feedback from OLs on feed additive activity

Number of samples analysed for feed additives in last 12 months?

Which analytes?

Has there been any issues with the methods used?

Are there any methods for other additives that you require support with?



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Review of Retained EU Regulation 152/2009 Laying down the methods of sampling and analysis for the official control of feed

Review of Retained EU Regulation 152/2009

ANNEX III - METHODS OF ANALYSIS TO CONTROL THE COMPOSITION OF FEED MATERIALS AND COMPOUND FEED

	Technique
A Determination of moisture	Drying and weighing
B Determination of moisture in animal and vegetable fats and oils	Drying and weighing
C Determination of the content of crude protein	Digestion, distillation, titration
D Determination of urea	Suspension in water, reaction with 4-DMAB, measurement of optical density
E Determination of volatile nitrogenous bases	1. Extraction, microdiffusion, titration 2. Extraction, distillation, titration
F Determination of amino acids (except tryptophan)	Extraction and ion-exchange chromatography with post-column derivatisation, photometric detection (amino acid analyser or HPLC system)
G Determination of tryptophan	Hydrolysis, extraction, HPLC-FL
H Determination of crude oils and fats	Acid digestion, solvent extraction, weighing
I Determination of crude fibre	Acid/alkaline digestion, filtrations, ashing, weighing
J Determination of sugar	Extraction, titration (Luff-Schoorl method)
K Determination of lactose	Extraction, fermentation, titration (Luff-Schoorl method)
L Determination of starch	Extraction, polarimetry
M Determination of crude ash	Ashing, weighing
N Determination of ash which is insoluble in hydrochloric acid	Acid digestion, ashing, weighing
O Determination of carbonates	Dissolution, measurement
P Determination of total phosphorus	Dry combustion or acid digestion, spectrophotometry
Q Determination of chlorine from chlorides	Extraction, precipitation, titration

Review of Retained EU Regulation 152/2009

ANNEX IV - METHODS OF ANALYSIS TO CONTROL THE LEVEL OF AUTHORISED ADDITIVES IN FEED

	Technique
A Determination of vitamin A	Hydrolysis, liquid liquid extraction, HPLC
B Determination of vitamin E	Hydrolysis, liquid liquid extraction, HPLC
C Determination of the trace elements iron, copper, manganese and zinc	Dry ashing, atomic absorption spectrometry
D Determination of halofuginone	Extraction, liquid liquid extraction, HPLC-UV
E Determination of robenidone	Extraction, column clean-up, HPLC-UV
F Determination of diclazuril	Extraction, SPE, HPLC-UV
G Determination of lasalocid sodium	Extraction, HPLC-FL

Review of Retained EU Regulation 152/2009

ANNEX V - METHODS OF ANALYSIS TO CONTROL UNDESIRABLE SUBSTANCES IN FEED

		Technique
A	Determination of free and total gossypol	Extraction, spectrophotometry
B	Determination of the levels of dioxins (PCDD/PCDF) and PCBs	Bioanalytical / GC-MS / GC-HRMS / GC-MS/MS

ANNEX VI - METHODS OF ANALYSIS FOR THE DETERMINATION OF CONSTITUENTS OF ANIMAL ORIGIN FOR THE OFFICIAL CONTROL OF FEED

		Technique
A	Light microscopy	Light microscopy
B	PCR	Polymerase chain reaction



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Review of Retained EU Regulation 152/2009

ANNEX VII - METHOD OF CALCULATING THE ENERGY VALUE OF POULTRYFEED

		Technique
A	Calculation and expression of energy value	Calculation

ANNEX VIII - METHODS OF ANALYSIS TO CONTROL ILLEGAL PRESENCE OF NO LONGER AUTHORISED ADDITIVES IN FEED

		Technique
A	Determination of methyl benzoate	Extraction, liquid liquid extraction, column clean-up, HPLC-UV
B	Determination of olaquinox	Extraction, HPLC-UV
C	Determination of amprolium	Extraction, HPLC-UV
D	Determination of carbadox	Extraction, HPLC-UV



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Review of Retained EU Regulation 152/2009

Of the 33 methods in 152/2009, the following 6 (18 %) were said to be used by all 4 respondents, either frequently or occasionally:

ANNEX III Methods of analysis to control the composition of feed materials and compound feed

- A. Determination of moisture
- C. Determination of the content of crude protein
- H. Determination of crude oils and fats
- I. Determination of crude fibre
- M. Determination of crude ash

ANNEX IV Methods of analysis to control the level of authorised additives in feed

- C. Determination of the trace elements iron, copper, manganese and zinc



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Review of Retained EU Regulation 152/2009

Are the methods being used?

If the methods are not being used, why not? Issues with the methods? Samples not being taken / analyses not being requested?



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Thank you for your attention.

Kirstin Gray

kirstin.gray@lgcgroup.com

Paul Hancock

paul.hancock@lgcgroup.com

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