National Reference Laboratory Feed Additives – Control and Authorisation

End of Year Report
2020 - 2021

Report reference: CP-2021-02

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Date: March 2021

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1. Introduction

Regulation (EC) No. 882/2004 *on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules* established a network of European and National Reference laboratories. Regulation (EC) No 882/2004 was repealed with effect from 14 December 2019 and replaced by Regulation (EU) 2017/625 *on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products*. Regulation 2017/625 supplements Regulation (EC) No 178/2002 *laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety*, aims for a high level of:

- protection of human, animal and plant health and of the environment via veterinary and phytosanitary measures;
- consumer protection in the internal market; and
- animal welfare along the agri-food chain.

In each area of food and feed control an EU Reference Laboratory (EURL) is identified to coordinate activities in that area. They are supported by a network of National Reference Laboratories (NRLs) which co-ordinate activities within their own member state and contribute to the European wide activities. NRLs are nominated by the Competent Authorities in the respective Member states. In the UK, the Competent Authority for feed additives is the Food Standards Agency (FSA).

The duties of the EURLs and NRLs are set out in legislation however their principal role is to provide analytical and scientific support to ensure that food and feed control is carried out effectively and in a harmonised manner, across the EU member states.

Article 94 of Regulation (EU) 2017/625 describes the responsibilities and tasks of EURLs as follows:

> 1. EURLs shall contribute to the improvement and harmonisation of methods of analysis, test or diagnosis to be used by official laboratories designated in accordance with Article 37(1) and of the analytical, testing and diagnostic data generated by them.
>
> 2. EURLs designated in accordance with Article 93(1) shall be responsible for the following tasks insofar as they are included in the reference laboratories' annual or multiannual work programmes that have been established in conformity with the objectives and priorities of the relevant work programmes adopted by the Commission in accordance with Article 36 of Regulation (EU) No 652/2014:
>
> (a) providing NRLs with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods;
>
> (b) providing reference materials to NRLs;
>
> (c) coordinating the application by the NRLs and, if necessary, by other official laboratories of the methods referred to in point (a), in particular, by organising regular inter- laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and the Member States of the results and follow-up to the inter-laboratory comparative testing or proficiency tests;
>
> (d) coordinating practical arrangements necessary to apply new methods of laboratory analysis, testing or diagnosis, and informing NRLs of advances in this field;

(e) conducting training courses for staff from NRLs and, if needed, from other official laboratories, as well as of experts from third countries;

(f) providing scientific and technical assistance to the Commission within the scope of their mission;

(g) providing information on relevant national, Union and international research activities to NRLs;

(h) collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA), the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC);

(i) assisting actively in the diagnosis of outbreaks in Member States of foodborne, zoonotic or animal diseases, or of pests of plants, by carrying out confirmatory diagnosis, characterisation and taxonomic or epizootic studies on pathogen isolates or pest specimens;

(j) coordinating or performing tests for the verification of the quality of reagents and lots of reagents used for the diagnosis of foodborne, zoonotic or animal diseases and pests of plants;

(k) where relevant for their area of competence, establishing and maintaining:

(i) reference collections of pests of plants and/or reference strains of pathogenic agents;

(ii) reference collections of materials intended to come into contact with food used to calibrate analytical equipment and provide samples thereof to NRLs

(iii) up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents; and

(l) where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods of analysis, testing or diagnosis of high standards.

3. EURLs shall publish the list of the NRLs designated by the Member States in accordance with Article 100(1).

Article 101 of Regulation (EU) 2017/625 describes the responsibilities and tasks of NRLs as follows:

(a) collaborate with the EURLs, and participate in training courses and in inter-laboratory comparative tests organised by these laboratories;

(b) coordinate the activities of official laboratories designated in accordance with Article 37(1) with a view of harmonising and improving the methods of laboratory analysis, test or diagnosis and their use;

(c) where appropriate, organise inter-laboratory comparative testing or proficiency tests between official laboratories, ensure an appropriate follow-up of such tests and inform the competent authorities of the results of such tests and follow-up;

(d) ensure the dissemination to the competent authorities and official laboratories of information that the EURL supplies;

(e) provide within the scope of their mission scientific and technical assistance to the competent authorities for the implementation of MANCPs referred to in Article 109 and of coordinated control programmes adopted in accordance with Article 112;

(f) where relevant, validate the reagents and lots of reagents, establish and maintain up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents;

(g) where necessary, conduct training courses for the staff of official laboratories designated under Article 37(1); and

(h) assist actively the Member State having designated them in the diagnosis of outbreaks of foodborne, zoonotic or animal diseases or of pests of plants and in case of non-compliance of consignments, by carrying out confirmatory diagnoses, characterisation and epizootic or taxonomic studies on pathogen isolates or pest specimens.

LGC currently holds the NRL roles for feed additives – control and authorisation. 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 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.

Technological additives, sensory additives, nutritional additives and zootechnical additives have sub-divisions of functional groups to which the feed additives are allocated, as applicable. As a result of technological and scientific development there were various substances that may have a technological effect on feed which was not described in relation to any of the original functional groups. For this reason, in June 2019, a new generic functional group called 'other technological additives' within the category 'technological additives' was created. At the same time a new functional group called 'physiological condition stabilisers' was created in the category 'zootechnical additives'. In addition to good farming practices ensuring the wellbeing of animals and the respect of animal welfare provisions in the EU, scientific studies show that some feed additives may help animals in good health to keep a good physiological condition, to contribute to animal welfare, to favourably affect the animal resilience to stress factors or to support their wellbeing in certain situations. Since the main function of these feed additives could not be allocated to any of the specific functional groups provided for in Regulation (EC) No 1831/2003, it was appropriate to create a new functional group within the category 'zootechnical additives'. The above additions to the functional groups came into force with Commission Regulation (EU) 2019/962. The full list of functional groups within each of the catogories, as of June 2019, are as follows:

1. In the category 'technological additives', the following functional groups are included:

    (a) preservatives: substances or, when applicable, micro-organisms which protect feed against deterioration caused by micro-organisms or their metabolites;

    (b) antioxidants: substances prolonging the storage life of feedingstuffs and feed materials by protecting them against deterioration caused by oxidation;

    (c) emulsifiers: substances that make it possible to form or maintain a homogeneous mixture of two or more immiscible phases in feedingstuffs;

    (d) stabilisers: substances which make it possible to maintain the physico- chemical state of feedingstuffs;

    (e) thickeners: substances which increase the viscosity of feedingstuffs;

    (f) gelling agents: substances which give a feedingstuff texture through the formation of a gel;

    (g) binders: substances which increase the tendency of particles of feedingstuffs to adhere;

    (h) substances for control of radionucleide contamination: substances that suppress absorption of radionucleides or promote their excretion;

    (i) anticaking agents: substances that reduce the tendency of individual particles of a feedingstuff to adhere;

    (j) acidity regulators: substances which adjust the pH of feedingstuffs;

    (k) silage additives: substances, including enzymes or micro-organisms, intended to be incorporated into feed to improve the production of silage;

    (l) denaturants: substances which, when used for the manufacture of processed feedingstuffs, allow the identification of the origin of specific food or feed materials;

    (m) substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action;

(n) hygiene condition enhancers: substances or, when applicable, microorganisms which favourably affect the hygienic characteristics of feed by reducing a specific microbiological contamination;

(o) other technological additives: substances or, when applicable, microorganisms added to feed for a technological purpose and which favourably affect the characteristics of the feed.

2. In the category 'sensory additives', the following functional groups are included:

(a) colourants:

(i) substances that add or restore colour in feedingstuffs;

(ii) substances which, when fed to animals, add colours to food of animal origin;

(iii) substances which favourably affect the colour of ornamental fish or birds;

(b) flavouring compounds: substances the inclusion of which in feedingstuffs increases feed smell or palatability.

3. In the category 'nutritional additives', the following functional groups are included:

(a) vitamins, pro-vitamins and chemically well-defined substances having similar effect;

(b) compounds of trace elements;

(c) amino acids, their salts and analogues;

(d) urea and its derivatives.

4. In the category 'zootechnical additives', the following functional groups are included:

(a) digestibility enhancers: substances which, when fed to animals, increase the digestibility of the diet, through action on target feed materials;

(b) gut flora stabilisers: micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora;

(c) substances which favourably affect the environment;

(d) other zootechnical additives;

(e) physiological condition stabilisers: substances or, when applicable microorganisms, which, when fed to animals in good health, favourably affect their physiological condition, including their resilience to stress factors.

Feed additives play an important role in animal nutrition, addressing various aspects such as feed safety, reduction of environmental emissions and sustainability in livestock farming. Before placing feed additives on the market, authorisation must be obtained as specified in Regulation (EC) No 1831/2003.

Pursuant to Regulation (EC) No 1831/2003, a list of the feed additives currently permitted for use in the EU can be found in the European Union Register of Feed Additives. The latest edition can be found at:

https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en (accessed 26 March 2021)

On 31st December 2020 all of the EU Regulations described above were transferred into the UK as EU retained law.

As feed additives are regulated products, folllowing Great Britain's exit from the EU all new feed additives will 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.

Guidance on the authorisation process for feed additives can be found on the FSA website at: https://www.food.gov.uk/business-guidance/regulated-products/feed-additives-guidance (accessed 26 March 2021)

The register of feed additives which lists the feed additives permitted for use in GB can also be found on the FSA's website at https://data.food.gov.uk/regulated-products (accessed 26 March 2021)

This report provides an update for the National Reference Laboratory role for Feed Additives – Control and Authorisation for the year April 2020 to March 2021.

2. EURL Proficiency Test 2020

The EURL's aim was to carry out one PT for the determination of vitamin D_3 in 2020. Originally it was planned that the samples of poultry feed would be dispatched in the April – May period but the PT was postponed until 2021 due to the Covid-19 pandemic. As the UK has now exited the EU, LGC as the GB NRL will no longer be able to participate in schemes and trials for NRLs organised by the EURL.

3. Additional Proficiency Tests

As we are no longer able to participate in proficiency tests (PT) organised by the EURL, alternatives have been investigated, however there is a general lack in available proficiency tests involving feed additives. As in previous years, vitamin E was determined in a FAPAS pig ration PT round and a z-score of 0.9 was obtained, zinc was analysed in the same sample and a z-score of 2.0 was obtained. Feed samples were also analysed for proximates (moisture, fat, protein and ash), elements (arsenic, cadmium, lead, mercury, nickel) and chloramphenicol and acceptable results were reported for all analytes.

Even though the number and range of PT's involving feed and feed additives are limited, the techniques which would be employed are the same or similar to those used for food. To ensure that a wide range of techniques is covered by our PT participation, the analyses undertaken are broken down by technique and are reviewed regularly to make sure that the most relevant techniques are covered. Table 1 presents a summary of some of the recent FAPAS rounds we have participated in and the techniques involved. The analyses in table 1 illustrate some of those carried out by the Office of the Government Chemist team, other teams within LGC participate in additional schemes and trials using different techniques such as PCR.

Start Date	Proficiency Test	Matrix	Analytes	Z-score	Enzymatic Hydrolysis	Acidic/ Basic Hydrolysis	Reflux inc saponification & Soxhlet, KD	Liquid liquid extraction	IAC	Microwave Digestion	Ashing (Muffle furnace)	Drying/ gravimetric	Derivatisation	Colorimetry/ Spectrophoto-metry	HPLC-FL	HPLC-UV	Post-column derivisation	ICP-OES	ICP-MS	LC-MSMS	Nitrogen analyser	ELISA	Distillation	Titration
05/02/2020	2986	Beef	Chicken	Satisfactory																		X		
05/02/2020	2986	Beef	Horse	Agrees																		X		
05/02/2020	2986	Beef	Lamb	Satisfactory																		X		
03/06/2020	10170	Pig ration	Moisture	2.4								X												
03/06/2020	10170	Pig ration	Ash	0.3							X	X												
03/06/2020	10170	Pig ration	Oil / Fat	-0.2		X	X					X												
03/06/2020	10170	Pig ration	Protein	0.3																	X			
03/06/2020	10170	Pig ration	Vitamin E	0.9			X	X						X	X	X								
03/06/2020	10170	Pig ration	Zinc	2.0						X								X						
06/07/2020	04390	Hazelnut	Aflatoxin B1	1.6					X						X		X							
06/07/2020	04390	Hazelnut	Aflatoxin B2	1.4					X						X		X							
06/07/2020	04390	Hazelnut	Aflatoxin G1	0.6					X						X		X							
06/07/2020	04390	Hazelnut	Aflatoxin G2	0.9					X						X		X							
06/07/2020	04390	Hazelnut	Total aflatoxins	1.4					X						X		X							
06/07/2020	04390	Hazelnut	Ochratoxin A	0.7					X						X									
02/09/2020	12100	3% Acetic acid	Formaldehyde (chromotropic acid method)	0.0										X										X
15/09/2020	21122	Liquid vitamin supplement	Vitamin B1	-1.2	X	X							X	X	X									
15/09/2020	21122	Liquid vitamin supplement	Vitamin B2 (total)	0.6	X	X							X	X	X									
02/10/2020	02411	Poultry feed	Chloramphenicol	-0.1				X												X				

19/10/2020	02412	Prawns	Nitrofuran metabolites - SEM (bound)	-0.4		X		X					X							X				
19/10/2020	02412	Prawns	Nitrofuran metabolites - SEM (total)	0.2		X		X					X							X				
04/11/2020	03163	Tonic water	Benzoic acid	0.1												X								
04/11/2020	03163	Tonic water	Acesulfame-k	2.7												X								
04/11/2020	03163	Tonic water	Aspartame	0.4												X								
26/11/2020	07385	Animal feed	Arsenic (total)	0.5						X									X					
26/11/2020	07385	Animal feed	Cadmium	0.0						X									X					
26/11/2020	07385	Animal feed	Lead	-0.1						X									X					
26/11/2020	07385	Animal feed	Mercury (total)	0.3						X									X					
26/11/2020	07385	Animal feed	Nickel	-0.2						X									X					
30/11/2020	20178	Dried apple (slurry)	Sulphur dioxide	1.2																			X	X

Table 1: Examples of proficiency tests participated in throughout 2020

4. Urea

The scope of the official method for the determination of urea in Commission Regulation (EC) No 152/2009 states that the 'method makes it possible to determine the level of urea in feed' however, discussions have been held throughout Europe as to whether the Regulation method is applicable to all types of feed. Urea (carbamide) as a feed additive is currently only authorised in feed for ruminants (Commission Implementing Regulation (EU) No 839/2012). The principle of the Regulation method is the sample is suspended in water with a clarifying agent. The suspension is filtered. The urea content of the filtrate is determined after the addition of 4-dimethylaminobenzaldehyde (4-DMAB) by measuring the absorbance at a wavelength of 420 nm. No validation or precision data is given in the Regulation method.

Towards the end of 2019 the EURL organised a trial for the determination of urea in 3 samples of non-ruminant complete feed (pet feed). The final report of the trial has now been published, a copy of which can be provided on request. The conclusion of the trial was that, for the pet food samples examined in the trial, the technique that provided the best results across the three test materials was LC-MS/MS, whereas spectrophotometry systematically overestimated the results in all the test materials. This conclusion confirmed the results obtained by LGC were are presented in Table 2 for information.

Sample	Technique	Result reported (mg/kg)	Assigned value	Z-score
J	Spectrophotometry	4530	18.36 +/- 0.99	1228
	LC-MS/MS	17.2		-0.3
C	Spectrophotometry	3289	249.2 +/- 6.6	61
	LC-MS/MS	318		1.4
G	Spectrophotometry	4073	935 +/- 24	17
	LC-MS/MS	969		0.2

Table 2: LGC's results from 2019 – 2020 EURL urea in pet food trial

5. Feed Additive Authorisation

From 1st January 2021 all new feed additives require authorisation before they can be used in Great Britain. As part of the authorisation process, the NRL Feed additives undertakes a scientific evaluation of the analytical methodology documentation submitted by the applicant, for feed additive authorisations, defined as being:

- A new feed additive
- A new use of an existing feed additive
- An existing feed additive
- Under a change in terms of existing feed additive
- A renewal of authorisation

Over the last few months extensive preparations have been made to enable the review of the methods for use in official control submitted by applicants to be reviewed efficiently. This set-up has included drafting quality documents and procedures, and in discussion with the FSA how the process is best carried out.

For more information on the authorisation process and the applications reviewed see LGC report CP-2021-03, Feed additive authorisation NRL report January – March 2021.

6. OCL Training

Paul Hancock joined LGC in July 2020 as Head of The Office Government Chemist. As part of his responsibilities, Paul has taken over the role of training officer for the Association of Public Analysts (APA) from Michael Walker. As training officer, Paul attends the regular APA training committee meetings and, in addition to e-mail contact, is available for Agriculture Analysts and Official Control Laboratories to request help or advice relating to the analysis of feed additives. No official training has yet been carried out this year, in part due to the social distancing restrictions in place due to COVID19.

7. Meetings and advice

While it has not been possible to have any physical meetings this year a number of virtual meetings were held with the FSA throughout the year to provide updates on the NRL position and to discuss the implications of EU exit. LGC also participated in the FSA's NRL EU exit survey and NRL review. Several meetings were also held to discuss the implementation of the process to assess feed additive authorisation applications following the end of the transition period and EU exit.

8. EURL Workshops

The 20th and 9th Annual Workshops (authorisation & control activities, respectively) of the European Union Reference Laboratory for Feed Additives (EURL-FA) and the consortia of National Reference Laboratories (NRLs) were organised this year as a joint webinar, held on 24th and 25th November 2020. The event was attended by 65 participants representing 26 National Reference Laboratories (NRLs) (including Norway and Switzerland), DG SANTE, EFSA and the EURL-FA.

The agenda was as follows:

24 November 2020

09:30 Virtual registration – Practical info on technical issues

10:00 Opening of the workshop: Welcoming & Introduction *Hendrik Emons & Christoph von Holst*

10:30 EURL-FA Control Activities in 2020 *Ursula Vincent*

11:00 Break

11:30 Research on issues regarding coccidiostats analysis at additive levels *Erik de Lange*

12:00 Identification of feed additives using DART *Christoph von Holst*

12:30 Lunch break

14:00 Register of Feed Additives – A guided tour *Almudena Rodríguez Sánchez-Beato*

14:30 Vitamin A – Is everything different now? *Irmengard Strnad*

15:00 Break

15:30 Ethoxyquin – an analytically challenging former feed additive *Robin Ørnsrud*

16:00 Review of Regulation (EU) 152/2009 – Current status *Frans Verstraete*

16:30 End of the 1st day

25 November 2020

10:00 EURL-FA activities 2020 (Authorisation) *Zigmas Ezerskis*

10:30 The validation of a method of analysis of Ethoxyquin residues in animal feed using Quechers and GC-MS/MS and the results obtained in our official control samples *Pilar Rodríguez Martínez*

11:00 Break

11:30 Presentation & discussion - Results from the ILC on urea (MVS UREA-19/01) and Results from the PT on urea *Ursula Vincent, Carlos Gonçalves*

12:30 Lunch break

14:00 FEEDAP panel: how is it working? *Angelica Amaduzzi*

14:30 Update from EFSA *Matteo Innocenti*

15:00 Break

15:30 Innovation in the feed additives Regulation *Marta Ponghellini*

16:00 Identification of some probiotics with pulsed field gel electrophoresis (PFGE): Results of a validation study *Atte von Wright*

16:30 Control activities of the EURL-FA activities in 2021 *Ursula Vincent*

16:50 End of the Webinar

Points of interest were:

Ethoxyquin
A presentation was given by *Robin Ørnsrud from the* Institute of Marine Research, Norway, on challenges with the former feed additive and a presentation on the validation of a method for the determination of ethoxyquin residues using QuEChERS and GC-MS/MS was given by Pilar Rodriquez from the Spanish NRL (Laboratori Agroalimentari Departament d'Agricultura, Ramaderia, Pesca i Alimentació).

Ethoxyquin (EQ) has, for decades, been widely used as an antioxidant in the feed sector, primarily in the marine industry and is used as an antioxidant, for example to inhibit the oxidation of highly unsaturated fatty acids in fishmeal and fish silage. In June 2017, the EU commission suspended the authorisation of ethoxyquin for all animal species and categories (Commission Implementing Regulation (EU) 2017/962).
The Institute of Marine Research, Norway, has performed surveillance and research on EQ since 2001. Analysis of fish feed and feed ingredients has shown the presence of EQ primarily in fish meal and fish feed, but other marine ingredients such as krill meal may also contain relatively high concentrations. Plant based ingredients such as rapeseed oil, soy protein concentrate and wheat flour may also contain EQ, but in lower concentrations than marine ingredients. Analyses from 2019 showed a more than 90% decline in EQ concentrations for fish feed and fish meal, indicating that the suspension of the authorisation of EQ has had the intended effect on the market.

Following the initial presentation on ethoxyquin, there was a brief discussion which included requests for clarification on whether or not a decision has been taken for establishing a value for the limit of detection of p-phenetidine (pPN) in the light of the ban of ethoxyquin (EQ) as a feed additive. Questions were also raised over the establishment of a threshold level for EQ, which would allow unavoidable carry-over to be differentiated from the illegal use of EQ in feed. DG SANTE clarified that the topic had not yet been formally discussed due to a lack of information on how to steer the discussion. The setting of a maximum permitted limit for EQ, above which addition would be assumed, is further complicated due to EQ decomposing in feed making it difficult to differentiate whether low levels of EQ may be due to cross-contamination or are the residual concentration of an higher added quantity. One possible solution is to screen for degradation compounds of EQ.

A question was also asked about the stability of the standard substances and the presenter explained that the standards are very unstable (e.g. a substantial interconversion occurred between quinone imine and de-ethylated EQ). The main transformation product of EQ in feed after several weeks' storage is the EQ dimer (EQDM) which exhibits a relatively high antioxidant activity and is stable. At the Institute of Marine Research, the policy is to prepare fresh EQ standard solutions daily. Deuterated standards are available, for example from Dr Ehrenstorfer, but there can be issues with conversion.

Discussions took place on how NRLs and official control laboratories should deal with EQ, with regards to the concentration in feed which should be considered as non-compliant. The following was proposed by the representative of DG SANTE:
• Both EQ and EQDM levels to be monitored;
• A reasonable LOQ that takes into consideration possible cross-contamination, should first be set;
• A feed would be considered compliant, and the residual content attributed to the unavoidable cross contamination if the concentration of EQ and / or EQDM was determined to be <LOQ;
• Conversely, a feed would be considered non-compliant if, on the contrary, the mass fraction of EQ and / or EQDM would be higher than the selected
threshold, then the feed would be considered as non-compliant if the concentration of EQ and / or EQDM was determined to be >LOQ, implying unauthorised use of EQ.

Another representative from DG SANTE enquired whether the LOD for the analysis of pPN in feed had been established. The EURL-FAC replied that the current target mass fraction value is 2.5 µg/kg in fish meal and single-laboratory validation is on-going at the EURL-FAC. When single-laboratory validation is complete for both fish meal and fish feed, an inter laboratory comparison trial will be organised to establish method performance characteristics. It is anticipated that the LOD recommended to the regulator, could be higher than the abovementioned target, based on the results and findings from the trial.

The second presentation described the development of a method to determine EQ in animal feed using a modified QuEChERS protocol with an ascorbic acid buffer to minimise EQ degradation. The extracts were analysed by gas chromatography coupled to a triple quadrupole mass spectrometry (GC-QQQMS). Triphenyl phosphate is used as an internal standard and quantification is carried out as the sum of the transformation products. The method was validated according to European Commission guidelines and gave excellent results in terms of trueness (recoveries 70-120%), intermediate precision (RSD < 20 %) and linearity (R2 ≥ 0.99) at the studied concentrations, as well as excellent sensitivity and selectivity. Following the presentation, the analytical problems related to the determination of EQ in feed with a high fat content were discussed as the extraction efficiency may be affected by high fat content. It was said that blank samples are difficult to find possibly due to

ethoxyquin being used to stabilise vitamin premixes. It was commented that with the authorisation of EQ suspended it has, apparently, been difficult to find out from industry what is being used instead of EQ, possibly an increase use of ingredients high in vitamin E.

Coccidiostats

Coccidiostats are antiprozoal agents that act upon Coccidia parasites and are used as an additive in animal feed. Wageningen Food Safety Research (WFSR) had identified issues with the analysis of coccidiostats at additive level and had, therefore, validated a new method which was based on a method developed and interlaboratory validated by the JRC. The method involved extraction with acetonitrile:methanol:water (80:10:10 v:v:v) after which standard addition was carried out. The extracts were then analysed by LC-MS/MS. The performance data for the validation was not acceptable and the extraction procedure and LC/MS/MS conditions modified. The results indicated that unusually high recoveries were seen when a large number of coccidiostats were spiked/ analysed simultaneously however this was not consistently confirmed. The EURL said that they will follow-up discussions on the analysis of coccidiostats when applying single and multi-analyte methods as the representative from the Slovenian NRL also reported issues when analysing coccidiostats at cross-contamination level and that the situation worsened in the presence of more coccidiostats.

The representative for the Portuguese NRL also stated that they had had problems recently with narasin as they had recently changed supplier for their standards and the new standards gave different responses (up to 20 %) for different lots when analysed by LC-DAD with post-column derivatisation.

DART mass spectrometry

Regulations authorising the use of zootechnical feed additives and coccidiostats specify the conditions of use including the composition of the feed additives in terms of the content of the active substance and the excipients. While there are standard methods to determine the active substance in a product there may not be methods to accurately identify the feed additive, for example, if the feed additive is the specific one authorised and if it contains the correct composition of excipients, etc.

In the presentation the suitability of direct analysis in real time, DART, mass spectrometry for the classification of feed additives containing coccidiostats was discussed. The analytical procedure involves the rapid extraction of the samples with an organic solvent and subsequent measurement of the extracts with DART mass spectrometry, no separation by liquid chromatography is required. The resulting MS spectra ‘fingerprints’ for the different feed additive preparations are then subjected to multivariate statistics.
During the discussions, the low sample intake (100 mg) was questioned and it was explained that the use of such a low sample weight was deemed acceptable as the procedure was aimed at the analysis of feed additive preparations rather than compound feeds containing low levels of feed additives. It was also mentioned that DART MS could be useful in respects to authenticity.

Vitamin A

Since authorisation for the antioxidant ethoxyquin was suspended, EU Member States have seen an increasing number of results for vitamin A below the declared values. An intensive root cause analysis identified three potential causes:

• Changes in formulation of vitamin A due to the suspension of ethoxyquin may have affected stability. Antioxidants must be present in sufficient quantities to protect the stability of, *inter alia*, vitamin A.

• Changes in feed production and feed composition. The impact of shear forces, temperature, pressure, etc. have to be taken into account with regards to the stability of feed additives such as vitamins. An even more intensive treatment for improving feed hygiene should not lead to a loss of vitamin A. Particle size and distribution in feed may have also been affected by formulation changes.

• Analysis of modified products. Harmonised representative sampling is essential as the particle distribution of vitamin A in the feed has a direct impact on the precision of the results.

During the discussion, it was agreed that due to the many analytical problems reported, further discussions are needed in an attempt to overcome the issues. It was also noted that industry is aware of the problems related to the analysis of vitamin A and are trying to help resolve them.

Status of the review of Commission Regulation (EC) No 152/2009

The representative of DG SANTE presented a detailed overview of the status of the revision of Commission Regulation (EC) No 152/2009, in particular with regards to the determination of several substances either classified as undesirable substances (e.g. gossypol) or as active substances in feed additives (e.g. several coccidiostats).

A brief discussion followed the presentation on the different performance criteria and tolerances that will be included in the updated Regulation for the same analyte using single and multi-analyte methods. It was questioned how these criteria should be applied for compliance with minimum or maximum limits. It was proposed by some of the NRLs that a method with the smallest measurement uncertainty should be prioritised for official control purposes, however, the representative of DG SANTE stated that any method fulfilling a criterion for reproducibility not exceeding 25 % is fit-for-purpose. However, it might be possible that disputes between operators and authorities concerning compliance or noncompliance of samples when using the single and multi-analyte methods may occur.

Urea

Presentations were given on the outcome of two studies conducted by the EURL-FAC on the interlaboratory comparison for the validation of the method on the determination of urea in ruminant feed and the proficiency test for the determination of urea in pet feed.

Urea is a feed additive authorised in ruminant feed by Commission Implementing Regulation (EU) No 839/2012 with an authorised maximum content in compound feed of 8800 mg kg^{-1}. Commission Regulation (EC) No 152/2009 describes the official method for the determination of urea in feed but does not specify the type of feed for which the method is applicable. Following reports from some of the NRLs stating that the official spectrophotometric method is not fit for purpose for feed other than ruminant feed and given that no method performance characteristics are provided, DG SANTE requested the JRC to provide an opinion on whether the current method should be completely removed from Commission Regulation (EC) No 152/2009 or if its scope should be restricted to ruminant compound feed. Additionally, provision of data on suitable methods for the determination of urea in feeds other than ruminant feed was requested. One requirement for suitability was that the method should have a LOQ of 100 mg kg^{-1} or less.

The outcome of the two studies concluded that Commission Regulation (EC) No 152/2009 should be revised restricting the scope of the official spectrophotometric method to ruminant compound feed only.
The following performance characteristics were established for the official method:

- the precision (RSD_R) in all cases, except for sheep feed at 3000 mg kg^{-1} measured at 420 nm, is better than 20 %;
- the method can be considered validated for the determination of urea in ruminant compound feed at authorised level;
- in this study the measurement wavelength, 420 nm or 435 nm, did not have a significant impact on the results;
- the trueness of the method is questionable for determining urea at mass fractions lower than 7000 mg kg^{-1}.

For the PT performed on pet feed:

- Only 36 % of the PT participants performed satisfactorily (according to the z score) in the analysis of the test material with the highest level of urea (935 mg kg^{-1}), 27 % for the intermediate level (249.2 mg kg-1) and 8.7 % for the lowest level (18.36 mg kg^{-1}). These scores indicate that the majority of the laboratories were unable to determine urea in pet feed accurately or that the method used was not appropriate for the type of samples analysed.
- The three most used analytical techniques were spectrophotometry, used by 45 % of the participants, LC-MS/MS, used by 23 %, and enzymatic-based methods used by 19 % of them.
- The technique that provided the best success rate across the three test materials was LCMS/MS, whereas spectrophotometry systematically overestimated the content in all the test materials.

FEEDAP panel

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) is one of EFSA's scientific panels and provides scientific advice on the safety and/or efficacy of additives and products or substances used in animal feed. When a company intends to market an additive in the EU, it must submit an application and a technical dossier with information on the additive, its conditions of use, control methods and data demonstrating its safety and efficacy. EFSA's FEEDAP Panel reviews this information and examines the efficacy and safety of the additive in terms of animal and human health as well as the environment. EFSA is assisted in this task by the EURL FA, which evaluates the analytical methods used to determine the presence of the additive in feed and its possible residues in food. The EURL report is an integral part of the scientific assessment. Without it, the opinion cannot be adopted and the European Commission will not authorise the additive. If the scientific evaluation has a favourable outcome, the European Commission prepares a draft regulation to authorise the additive.
Then, the discussions focussed on inconclusive EFSA opinions. It was mentioned that 40 % of all EFSA opinions on feed additives are inconclusive for at least one aspect (for example safety or efficacy). With regards to efficacy, it was noted that quite often EFSA was not able to come to a conclusion on efficacy as the corresponding studies had not been properly carried out. Generally, the revised studies, when carried out to EFSA's guidelines, resolved most of the issues and allow conclusions to be made on the efficacy of the feed additives. Concerning the safety, the inconclusive opinions were mostly the result of a lack of scientific knowledge in the field at the time of the evaluation. The EURL also admitted that occasionally no analytical methods suitable for feedingstuffs can be recommended for intrinsically complex feed additives such as botanically defined flavourings. Furthermore, EFSA pointed out that

feed additives and production technologies are constantly evolving, and therefore new problems regularly arise during dossier assessments.

Identification of some probiotics with pulsed field gel electrophoresis (PFGE)
In 2017 Biosafe Biological Safety Solutions Ltd (Biosafe) was commissioned by CEN to develop a standard for the pulsed field gel electrophoretic (PFGE) identification of probiotic microorganisms belonging to the genera *Lactobacillus*, *Pediococcus*, *Enterococcus* and *Bacillus*. The approach is based on the electrophoretic analysis of strain specific restriction patterns of bacterial genomic DNA. In PFGE, in contrast to standard electrophoretic conditions, the direction of the electric field alternates in short pulses making the separation of very large DNA fragments possible. The approach chosen by Biosafe was to design a generic method applicable to all these genera rather than focusing on the development of genus-specific procedures. The in-house method was finalized in 2019, and the validation trial involving nine laboratories was started in September 2019 and finished in May 2020.
The PFGE patterns obtained by the different partners displayed a high degree of consistency across the laboratories, and they clearly discriminated between the tested strains. It was concluded that the method can be used as a tool for strain-specific identification of probiotic bacteria. During the discussion, pros and cons of the PFGE method were highlighted and various alternatives for the strain identification of microorganism such as PCR and whole genome sequencing (WGS) were mentioned.

9. NRL Forward Workplan

The current contract for the NRL for feed additives comes to an end on 31st March 2021. Whilst the formal contract has yet to be completed, it is understood that LGC has been awarded the contract for the NRL for feed additives for Great Britain. Once the contract has been signed the workplan for 2021 will be drafted.

10. 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/ (accessed 25 March 2021)

It is our intention to review and further develop the website under the new contract.

11. Official Control Laboratory accreditation status

One of the NRLs roles is to maintain a list of the accreditation status of relevant OCLs. Table 3 presents the accreditation status concerning feed and feedingstuffs as of March 2021, according to the schedules published on the UKAS website. There appears to have been no significant changes to the laboratories' scopes since the last review in February 2020.

Laboratory	UKAS accreditation status - Feed additives
Hampshire Scientific Services	No reference to feed / feed additives apart from melamine in feed products containing milk or soya.
Kent Scientific Services	Accredited in animal feedingstuffs – Aflatoxins B1, B2, G1 and G2, ash content, citrinin, crude fibre, copper, inorganic arsenic, lead, cadmium, moisture, nitrogen, oil, vitamin A, vitamin E, total mercury, fumonisins B1 and B2, Ergot alkaloids (Ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergotamine), deoxynivalenol, T2 and HT2 toxins, histamine. Accredited in 'unspecified foods and animal feed' - Additives, colourings, preservatives and related contaminants and composition using generic in-house methods with the following techniques: HPLC, LC/MS, GC, GC/MS, AAS, UV VIS, spectrophotometry, microscopy, ELISA and wet chemistry (drying, weighing and titration).
Lancashire County Scientific Services	Accredited in animal feedingstuffs - Ash, crude oil and fat, fibre, moisture, protein (calculated value), vitamin A, vitamin E, cadmium, cobalt, copper, iron, lead, magnesium, manganese, zinc.
Public Analyst Scientific Services, Wolverhampton	No reference to feed / feed additives.
Aberdeen Scientific Services (Aberdeen City Council)	Accredited in animal feedingstuffs - Contaminants and composition (generic in-house procedures using GC, GC-MS, HPLC, AAS, ICP-OES, UV/Visible spectrophotometry, microscopy and classical techniques), Aflatoxins B1, B2, G1, G2 and total aflatoxins, arsenic and selenium, ash, calcium, magnesium, iron, copper, manganese, zinc, lead, cadmium, mercury, moisture, nitrogen, oil, protein, crude fibre, vitamin A, vitamin E.
Dundee City Council Scientific Service (Tayside Scientific Services)	Accredited in animal feedingstuffs - Compositional analysis, additives, colourings, preservatives and related contaminants (flexible scope using HPLC, GC, GC-MS and UV spectroscopy, gravimetric, titrimetric and other classical wet chemistry techniques), determination of elements (AAS and ICP-OES), foreign body identification, aflatoxins B1, B2, G1, G2, ochratoxin A, zearalenone, ash, crude fibre, moisture, oil (total), nitrogen, (crude) protein, vitamin A, vitamin E, cobalt, copper, iron, manganese, zinc, cadmium, lead, arsenic, selenium.
Edinburgh Scientific Services (The City of Edinburgh Council)	Accredited in animal feedingstuffs - Aflatoxins B1, B2, G1 and G2, ochratoxin A, ash, acid insoluble ash, crude fibre, oil / fat, moisture, nitrogen, protein, arsenic, cadmium, cobalt, copper, lead, mercury, selenium, zinc, vitamin A, vitamin E, isolation and confirmation of *Salmonella spp*, detection and identification of bacteria DNA using specific genomic sequences.
Glasgow Scientific Services	Accredited in feedingstuffs - Ash, crude fibre, crude oils and fats, moisture, nitrogen, protein, crude protein, cadmium, copper, lead, selenium, zinc, calcium, copper, iron, magnesium, manganese, phosphorus, vitamin A, vitamin E.
Minton, Treharne and Davies Limited	Accredited in animal feedingstuffs - ash, crude fibre, lead, cadmium, moisture, nitrogen, protein, oil, method development using AAS, HPLC, ELISA, titrimetry and gravimetry.

Table 3: Accreditation status of UK OCLs as of March 2021

12. Complimentary work

Food and feed legislation

Under the Government Chemist function, feed and food law is regularly reviewed and a report placed on the Government Chemist website describing recent changes to relevant law. The compendium of UK food and feed legislation with associated context and changes can be found at: https://www.gov.uk/government/organisations/government-chemist