Scientific Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP)

Minutes of the 96th Plenary Meeting

Held on 18-20 June 2013, Parma

(Agreed on 9 July 2013)

Participants

- **Panel Members**

  Gabriele Aquilina, Alex Bach, Vasileios Bampidis, Maria De Lourdes Bastos, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen,[1] Maria Saarela,[2] Kristen Sejrsen, Patrick van Beelen, John Wallace[3] and Johannes Westendorf.

- **Hearing Experts**

  N/A

- **European Commission**

  Marta Ponghellini[4]

- **EFSA**

  - **FEED Unit:** Claudia Roncancio-Peña, Jaume Galobart, Montserrat Anguita, Gloria López-Gálvez, Rosella Brozzi, Lucilla Gregoretti, Paola Manini, Maria Vittoria Vettori, Jordi Tarrés-Call, Nicola Jane Reynolds and Cecilia Lloyd.
  - **SCISTRAT Directorate:** Andras Szoradi[5]

- **Observers**[6]

  Ludovic Arnaud (FEFANA), Thomas Brenten (Mars GmbH), Heidi Burrows (AB Vista), Oliviero Costa (Ministero della Salute Italia), Juliane Dohms (CJ Europe GmbH), Didier Jans (FEFANA), Jean Kennedy (Alltech), Elinor Mc Cartney (Pen & Tec Consulting SL), Thomas Meyer (FEDIAF), Noriko Nakamura (Calpis Co., Ltd.), Michael Rasmussen (Novozymes A/S), Renato Rosà (EMFEMA), Lisa Saibi (Adisseo France SAS), Gerald Schultheis (FEFANA Working Group Mycotoxins), Giuseppe Simone (FEDIAF), Bert Soenen (Elanco), Piet Van Dijck (DSM), Davy Van Gaver (Huvepharma NV), Stefan Wittocx (Orffa International Holding BV)

1 Present only on 18 and 19 June.
2 Present only on 18 and 19 June.
3 Present only on 19 and 20 June.
4 Present only on 18 June.
5 Present only on 18 June.
6 Present only on 18 June for the session open to observers.

1. Welcome and apologies for absence

The Chair welcomed the participants, especially Fernando Ramos who has joined the Panel.

Apologies were received from Lucio Guido Costa.

The Chair welcomed all observes who attended the open session of the plenary. A tour de table followed the Chair's welcome to enable all meeting participants to introduce themselves

2. Adoption of agenda

The agenda was adopted after the deletion of the items "Canthaxanthin for chickens for fattening, chickens reared for laying, laying hens, salmon and trout, other poultry, other fish, petfood and other non food-producing animals (EFSA-Q-2009-00486)", "Rovabio® Excel (endo-1,3(4)-beta-glucanase and endo-1,4-beta-xylanase) for chickens for fattening, laying hens, turkeys for fattening, piglets (weaned), pigs for fattening, ducks, guinea fowls, quails, geese, pheasants, pigeons (EFSA-Q-2010-01287)" and "Brilliant black PN for all animal species (EFSA-Q-2010-01526)

3. Declarations of interest

In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes[7] and the Decision of the Executive Director implementing this Policy regarding Declarations of interests[8], EFSA screened the Annual declaration of interest and the Specific declaration of interest (SDoI) filled in by the experts invited for the present meeting. For further details on the outcome of the screening of the SDoI, as well as the Oral declaration of interests at the beginning of the meeting, please refer to Annex I.

4. Presentation of the 'Guidelines for Observers'

The background to the initiative to open selected plenary meetings to Observers was presented to the meeting together with the guidelines and a request that all participants provide feedback.

5. Agreement of the minutes of the 95th Plenary meeting held on 16-18 April 2013

The minutes of the 95th Plenary meeting were reviewed and agreed.[9]

6. Report on written procedures since 95th Plenary meeting

The scientific opinion on "Selenomethionine for all animal species (EFSA-Q-2011-01109)" was adopted by written procedure on 2 May 2013.[10]

7. Scientific outputs submitted for discussion and possible endorsement for public consultation

7.1. Guidance document for the renewal of the authorisation of feed additives (EFSA-Q-2012-00962)

The Chair of the working group (WG) presented the question and the draft opinion. The Panel through this self-task intends to produce a guidance for the assessment of applications for the renewal of the authorisation of feed additives.

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7 http://www.efsa.europa.eu/en/keydocs/docs/independencepolicy.pdf
8 http://www.efsa.europa.eu/en/keydocs/docs/independencerules.pdf
9 http://www.efsa.europa.eu/en/events/event/130416b-m.pdf
10 http://www.efsa.europa.eu/en/efsajournal/pub/3219.htm

The draft guidance was presented. The Panel endorsed the draft guidance document which will be subject to public consultation.[11]

7.2. Update of the guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA-Q-2013-00303)

The Chair of the WG presented the question and the draft opinion. The Panel through this self-task intends to update the guidance for the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition following the recent scientific developments.

The draft guidance was presented. The reasoning for updating the guidance is presented in Annex II. The Panel endorsed the draft guidance document which will be subject to public consultation.[12]

8. New Mandates

8.1. New applications under Regulation (EC) No 1831/2003

The Commission has forwarded to EFSA the following new applications of feed additives seeking authorisation under Regulation (EC) No 1831/2003 since the last Plenary meeting. These applications were presented to the Panel, who accepted them:

EFSA-Q-Number	Subject
EFSA-Q-2013-00406	Manganese hydroxychloride (IntelliBond® M) for all animal species
EFSA-Q-2013-00431	Natural mixture of dolomite plus magnesite and magnesium-phyllosilicates (Fluidol) for all animal species
EFSA-Q-2013-00407	Aminotrace Copper Bislysinate for all animal species
EFSA-Q-2013-00343	Deccox® (Decoquinate) for chickens for fattening
EFSA-Q-2013-00528	Enzy Carboplus®, Enzy Carboplus®L (preparation of xylanase and β-glucanase) for chickens for fattening, laying hens, turkeys for fattening, piglets (weaned), minor avian species (game birds, ducks, geese, pigeons, sporting and ornamental birds) including laying birds, chickens reared for laying, turkeys reared for breeding
EFSA-Q-2013-00522	Coxiril® (Diclazuril) for poultry, guinea fowl
EFSA-Q-2013-00529	Potassium ferrocyanide for all species

8.2. New questions under Regulation (EC) No 178/2002

EFSA-Q-Number	Subject
EFSA-Q-2013-00340	Erythrosine for cats and dogs, ornamental fish, reptiles
EFSA-Q-2013-00421	*Lactobacillus plantarum* ATCC 55058 and ATCC 55942 for all animal species
EFSA-Q-2013-00436	*Pediococcus acidilactici* CNCM I-3237 for all animal species

[11] http://www.efsa.europa.eu/en/consultations/call/130626.htm
[12] http://www.efsa.europa.eu/en/consultations/call/130626a.htm

8.3. Valid applications under Regulation (EC) No 1831/2003 since the previous meeting

Applications considered valid for the start of the assessment:

#	EFSA-Q-Number	Subject	Valid on
1	EFSA-Q-2013-00072	AviMatrix (Preparation of benzoic acid, calcium formate and fumaric acid) for chickens for fattening, chickens reared for laying, minor avian species for fattening and to point of lay	29/04/2013
2	EFSA-Q-2013-00090	Fumonisin esterase (FUMzyme®) for pigs	02/05/2013
3	EFSA-Q-2013-00022	Enzy Phostar® (6-phytase) for chickens for fattening, laying hens, turkeys for fattening, weaned piglets, pigs for fattening, sows for reproduction, minor avian species (game birds, ducks, geese, pigeons, sporting and ornamental birds), including laying birds, minor porcine species, chickens reared for laying, turkeys reared for breeding	03/05/2013
4	EFSA-Q-2012-00534	Lutein for laying hens, chickens for fattening, turkeys for fattening, other poultry for fattening and laying	06/05/2013
5	EFSA-Q-2012-00377	L-valine feed grade (ValAMINO) for all animal species	08/05/2013
6	EFSA-Q-2013-00069	Argile verte du Velay (Velay Green Clay) Natural mixture of illite, montmorillonite and kaolinite for all animal species	15/05/2013
7	EFSA-Q-2013-00002	Coenzyme Q10 (Kaneka Q10) for all animal species	16/05/2013
8	EFSA-Q-2012-00953	Vitamin B_2 (riboflavin) for all animal species	23/05/2013
9	EFSA-Q-2013-00343	Deccox® (Decoquinate) for chickens for fattening	29/05/2013
10	EFSA-Q-2012-01000	PEG castor oil (Glyceryl polyethyleneglycol ricinoleate) for all animal species	30/05/2013
11	EFSA-Q-2013-00205	MycoCell (*Saccharomyces cerevisiae* NCYC R404) for dairy cows for milk production	06/06/2013

These applications were assigned to the working groups on Organic acids (#1), Mycotoxin detoxifying agents (#2), Enzymes (#3), Colourings (#4), Amino acids (#5), Technological additives (#6 and #10), Vitamins (#7 and #8), Coccidiostats (#9) and Microorganisms (#11).

9. Feedback from the Scientific Committee/the Scientific Panel, Working Groups, EFSA, the European Commission

- A member of the FEED Unit informed on the status of the different call for tenders launched by the unit. The final report on "Review of substances/agents that have direct beneficial effect on the environment: mode of action and assessment of efficacy" has been delivered and is available on the EFSA website.[13]

[13] http://www.efsa.europa.eu/en/supporting/pub/440e.htm

- The Panel was informed about the questionnaires sent to stakeholders regarding the use of zinc in animal nutrition, in the context of a specific mandate on Revision of Maximum Content of Zinc in Feed. The questionnaires aim to collect data on industry recommendations regarding use of zinc in feed and typical feed compositions.
- The Panel was also informed on the Public consultation on the draft guidance of EFSA on emissions of plant protection products from protected crops (greenhouses and crops grown under cover).

10. Other scientific topics for information and/or discussion

Not discussed

11. Questions from Observers

The Chair granted the observers the opportunity to ask questions sent to EFSA in advance, which were answered by the FEEDAP Panel or the FEED Unit. The list of questions and answers are presented in Annex III.

12. Scientific outputs submitted for discussion and possible adoption[14]

12.1. Chemically defined flavourings from Flavouring Group 29 - Thiazoles, thiophene, thiazoline and thienyl derivatives for all animal species and categories (EFSA-Q-2010-01180)

The rapporteur presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 and the authorisation under Article 4 of Regulation (EC) No 1831/2003 of the chemically defined flavourings from Chemical Group 29 as sensory additives for all animal species. The current opinion concerns only 3-acetyl-2,5-dimethylthiophene.

The draft opinion was discussed. However, due to lack of quorum, the opinion will be submitted for possible adoption to the next plenary.

12.2. Sodium saccharin for pigs, piglets (suckling and weaned), pigs for fattening, calves for rearing and calves for fattening (EFSA-Q-2010-01228)

A member of the WG presented the question. This question refers to the re-evaluation under Article 10 and the authorisation under Article 4 of Regulation (EC) No 1831/2003 of sodium saccharin as sensory additives for pigs and calves for rearing and fattening.

The WG sought the advice of the Panel on some aspects of the safety assessment for this compound.

12.3. L-tyrosine for all animal species (EFSA-Q-2010-01312)

The rapporteur presented the question and the draft opinion. This question refers to the authorisation under Article 4 of Regulation (EC) No 1831/2003 of L-tyrosine as a nutritional additive for all animal species.

The draft opinion was discussed. The Panel concluded that l-tyrosine is safe for target animals, when used under the proposed conditions of use. Similarly, it is considered safe for consumers and the environment. This product should be considered as irritant

[14] During the scientific risk assessment process of each output, the relevant guidelines and guidance documents have been followed.

to skin and eyes and a skin sensitizer. The Panel also concluded that L-tyrosine is efficacious in cases where high requirements for tyrosine as melanin precursor occur.

The opinion was adopted.

12.4. Quinoline yellow for all animal species (EFSA-Q-2010-01523)

Not discussed due to lack of time.

12.5. Natugrain TS/L (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase) for pigs for fattening (EFSA-Q-2011-00061)

The Chair of the WG presented the question and the draft opinion. This question refers to the authorisation under Article 4 of Regulation (EC) No 1831/2003 of Natugrain TS/L (endo-1,4-beta-xylanase and endo-1,4-beta-glucanase) as a zootechnical additive for pigs for fattening.

The draft opinion was discussed. The safety for consumer, user and the environment as well as those aspects related to the genetic modification have been covered in a previous assessment and would not be affected by the requested extension of use. The Panel concluded that the additive is safe for pigs for fattening and has the potential to be efficacious.

The opinion was adopted.[15]

12.6. Potassium sorbate for all animal species except cats and dogs (EFSA-Q-2011-00841)

The rapporteur presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 of Regulation (EC) No 1831/2003 of potassium sorbate as a technological additive (silage additive) for all animal species except cats and dogs.

The draft opinion was discussed. The Panel concluded that potassium sorbate is safe for the target species when used at the maximum proposed concentration of 300 mg/kg forage. Similarly, it is considered safe for consumer and environment, but although it is not a skin sensitiser it is a skin and eye irritant and exposure by inhalation is considered hazardous. The Panel also concluded that potassium sorbate has the potential to improve aerobic stability of silage with a dry matter content of 21-38%.

The opinion was adopted.[16]

12.7. Vitamin D_3 for pigs, piglets, bovines, ovines, calves, equines, chickens for fattening, turkeys, other poultry, fish, other species or categories of animals (EFSA-Q-2011-00951)

The rapporteur presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 of Regulation (EC) No 1831/2003 of Vitamin D_3 as a nutritional additive for pigs, piglets, bovines, ovines, calves, equines, chickens for fattening, turkeys, other poultry, fish, other species or categories of animals.

The draft opinion was discussed. The Panel noted that for turkeys for fattening, equines, bovines, ovines and pigs the maximum content for vitamin D_3 does not provide any margin of safety, and that, except for pigs, the maximum content is above the upper safe level, according to the National Research Council data when fed for

15 http://www.efsa.europa.eu/en/efsajournal/pub/3285.htm
16 http://www.efsa.europa.eu/en/efsajournal/pub/3283.htm

more than 60 days. On the other hand, no safety concern is identified for the use of vitamin D_3 in chickens for fattening and fish. Notwithstanding the long history of supplementing compound feed with vitamin D and the absence of publicly reported intolerances, the FEEDAP Panel is not in the position to draw final conclusions on the safety of vitamin D and considers the current maximum contents as temporarily acceptable. The Panel concluded that vitamin D_3 is safe for the consumer and the environment. Moreover, the Panel considered it prudent to treat the vitamin D_3 under assessment as irritant to skin and eyes, and as skin sensitizer, and concluded that exposure to dust of the solid formulation is harmful. The vitamin D_3 under application is regarded as an effective dietary source of the vitamin in animal nutrition.

The opinion was adopted.

12.8. Biostrong® 510 (preparation of essential oil of thyme and star anise) for chickens and minor avian species for fattening and rearing to point of lay (EFSA-Q-2011-01152)

Not discussed due to lack of time.

12.9. *Pediococcus pentosaceus* (DSM 14021), *Pediococcus pentosaceus* (DSM 23688) and *Pediococcus pentosaceus* (DSM 23689) for all animal species (EFSA-Q-2012-00091)

The rapporteur presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 of Regulation (EC) No 1831/2003 of three strains of *Pediococcus pentosaceus* (DSM 14021, DSM 23688 and DSM 23689) as technological additives (silage additives) for all animal species.

The draft opinion was discussed. The Panel concluded that the three species are safe for the target species, consumers and the environment. The additives should be regarded as eye and skin irritants and as skin and respiratory sensitizers. The three strains have the potential to improve the ensiling process of easy and moderately difficult to ensile forages.

The opinion was adopted.[17]

12.10. Brilliant Blue FCF for cats and dogs (EFSA-Q-2012-00333)

The rapporteur presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 of Regulation (EC) No 1831/2003 of Brilliant Blue FCF as a sensory additive for cats and dogs.

The draft opinion was discussed. The Panel concluded that Brilliant Blue FCF is safe for cats and dogs at a maximum concentration of 278 and 334 mg/kg feed, respectively. The additive should be regarded as hazardous by inhalation and as irritant to skin and eyes, but not as a skin sensitizer. Brilliant Blue FCF is efficacious in colouring petfood. The Panel recommended setting a maximum content of 300 mg/kg complete feed.

The opinion was adopted.

[17] http://www.efsa.europa.eu/en/efsajournal/pub/3284.htm

12.11. Manganese amino acid chelate, hydrate for all animal species (EFSA-Q-2012-00436)

The Chair of the WG presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 of Regulation (EC) No 1831/2003 of manganese amino acid chelate, hydrate as a nutritional additive for all animal species.

The draft opinion was discussed. The Panel identified some issues that required further discussion and asked the WG to provide an updated draft to the next plenary meeting.

12.12. Manganous oxide for all animal species (EFSA-Q-2012-00439)

Not discussed due to lack of time.

12.13. Iron amino acid chelate, hydrate for all animal species (EFSA-Q-2012-00490)

The Chair of the WG presented the question and the draft opinion. This question refers to the re-evaluation under Article 10 of Regulation (EC) No 1831/2003 of iron amino acid chelate, hydrate as a nutritional additive for all animal species.

The draft opinion was discussed. The Panel concluded that when used up to the currently authorised maximum content of total iron in complete feed, the additive is considered safe for all animal species/categories, consumers and the environment. The additive should be considered as a skin, eye and respiratory irritant and a skin/respiratory sensitiser. Iron chelate of amino acids, hydrate, is considered an effective source of iron. The Panel recommended reducing the maximum iron contents in complete feed for bovines and poultry to 450 mg/kg and for pets to 600 mg/kg.

The opinion was adopted.[18]

12.14. AGal-Pro (alpha-galactosidase and endo-1,4-beta-glucanase) for chickens reared for laying, minor poultry species for fattening (EFSA-Q-2012-00909)

The Chair of the WG presented the question and the draft opinion. This question refers to the authorisation under Article 4 of Regulation (EC) No 1831/2003 of AGal-Pro (alpha-galactosidase and endo-1,4-beta-glucanase) as a zootechnical additive for chickens reared for laying, minor poultry species for fattening.

The draft opinion was discussed. The safety for consumer, user and the environment have been covered in a previous assessment and would not be affected by the requested extension of use. The Panel concluded that the additive is safe for the target species and efficacious.

The opinion was adopted.[19]

13. Any other business

- The Head of Unit provided feedback on the status of Regulation (EU) No 288/2013 which suspended the authorisation of the additive Toyocerin® (*Bacillus cereus* var. toyoi).
- The Panel also was informed about an erratum identified in the opinion regarding the product “Betaine in the form of betaine anhydrous and betaine hydrochloride for all animal species (EFSA-Q-2011-00259)” which was adopted during the last plenary. This erratum will be corrected in the opinion.

[18] http://www.efsa.europa.eu/en/efsajournal/pub/3287.htm
[19] http://www.efsa.europa.eu/en/efsajournal/pub/3286.htm

Annex I

Interests and actions resulting from the screening of Specific Declaration of Interests (SDoI)[20]

In the SDoI filled for the present meeting Dr. Alex Bach declared the following interest: Biostrong® 510 (preparation of essential oil of thyme and star anise) for chickens and minor avian species for fattening and rearing to point of lay (EFSA-Q-2011-01152). In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes and the Decision of the Executive Director implementing this Policy regarding Declarations of Interests, and taking into account the specific matters discussed at the meeting in question, the interest above was deemed to represent a conflict of Interest.

This results in the impossibility for the expert to be present when that item (Biostrong® 510 (preparation of essential oil of thyme and star anise) for chickens and minor avian species for fattening and rearing to point of lay (EFSA-Q-2011-01152)) is discussed, voted on or in anyway processed by that concerned scientific group.

In the SDoI filled for the present meeting Dr. John Wallace declared the following interest: *Pediococcus pentosaceus* (DSM 14021), *Pediococcus pentosaceus* (DSM 23688) and *Pediococcus pentosaceus* (DSM 23689) for all animal species (EFSA-Q-2012-00091). In accordance with EFSA's Policy on Independence and Scientific Decision-Making Processes and the Decision of the Executive Director implementing this Policy regarding Declarations of Interests, and taking into account the specific matters discussed at the meeting in question, the interest above was deemed to represent a conflict of Interest.

This results in the impossibility for the expert to be present when that item (*Pediococcus pentosaceus* (DSM 14021), *Pediococcus pentosaceus* (DSM 23688) and *Pediococcus pentosaceus* (DSM 23689) for all animal species (EFSA-Q-2012-00091)) is discussed, voted on or in anyway processed by that concerned scientific group.

[20] The Annual Declarations of Interests have been screened and approved before inviting the experts to the meeting, in accordance with the Decision of the Executive Director implementing the Policy on Independence regarding Declarations of Interests.

Annex II

The need to revise the Technical Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition

1. Introduction

The first guidance for applicants on how to assess toxigenic potential of species of the genus *Bacillus* was developed by the then Scientific Committee on Animal Nutrition (SCAN) and published in 2000 with the title *Opinion on the Safety of use of Bacillus species in Animal Nutrition* (EC, 2000). The SCAN guidance took as its basis the then existing knowledge on the structure and biogenesis of toxins produced by *B. cereus*, assuming that toxins found in other *Bacillus* species would have sufficiently similar properties to be detected by the methods developed for the *Bacillus cereus* group. Since the SCAN Opinion was published it became apparent that the few reports of *B. cereus*-like enterotoxins occurring in species other than those of the *B. cereus* group and cited in the SCAN Opinion were likely to have resulted from a misidentification of the strain involved (From et al., 2005). The few incidents of food poisoning investigated where non-*B. cereus* group strains were determined to be the causative organism suggested an association with heat-stable surfactins and similar cyclic lipopeptides with surfactin activity rather than the enterotoxins typical of *B. cereus*. As hazards of this nature were not considered in the original SCAN Opinion, the FEEDAP Panel undertook a revision, also taking the opportunity to adopt this revision document as part of its technical guidance provided to applicants seeking authorisation of feed additives (EFSA FEEDAP Panel, 2011). The data requirements proposed for species belonging to the *B. cereus* group in the revised opinion was essentially unchanged other than requiring a full genome sequence analysis. The bulk of the changes introduced involved a substantial revision of the sections dealing with species other than those of the *B. cereus* group, with a shift to the detection of a capacity for the production of surfactins.

2. Surfactins and related cyclic lipopeptides

Surfactins represent a family of structurally similar cyclic lipopeptides which possess potent surfactant activity (Figure 1). The biosynthesis of these microbial lipopeptides is accomplished non-ribosomally by large multienzyme systems that are composed of catalytic domains that catalyse all steps in peptide biosynthesis including the selection and ordered condensation of amino acid residues. It is know that these surfactins create pores in epithelial cells (From et al., 2007a; From et al., 2007b) and are toxic to sperm cells (Salkinoja-Salonen et al., 1999).

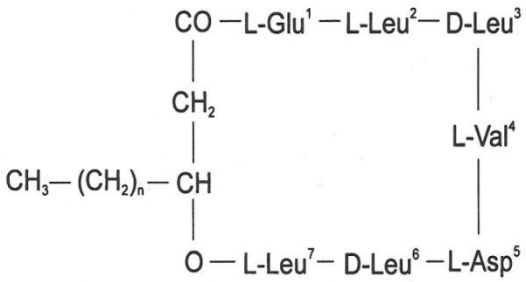

Figure 1: **Primary structure of surfactins (n = 9-11) (from Carrillo et al., 2003)**

Some examples of toxic peptides produced by *Bacillus* species are:

- amylosin produced by *B. amyloliquefaciens*, a member of the *B. subtilis* group (Mikkola et al., 2007);
- fengycin and surfactin from *B. subtilis* and *B. mojavensis* (Hwang et al., 2009, From et al., 2007a);
- pumilacidin from *B. pumilus* (From et al., 2007b);
- lichenysin from *B. licheniformis* (Nieminen et al., 2007).

Pumilacidin was associated with a foodborne poisoning outbreak linked to rice (From et al., 2007b). Lichenysin was produced by *Bacillus* sp. isolated from mastitis in dairy cows. Surfactin and amylosin were proposed to be the origin of the cytotoxic activities found in some strains of *B. mojavensis* implicated in foodborne poisoning (From et al., 2007a, Apetroaie-Constantin et al., 2009). All the above-described peptides have toxic activities on cell lines and sperm cells, as seen with cereulide, the emetic toxin of *B. cereus.* However, these toxins have a structure and biogenesis distinct from that of cereulide. They are lipopeptides which confer their surfactant properties (Ongena and Jacques, 2008).

Early data suggested that the surfactin-like cyclic peptides were produced by about 3-4 % of strains of *B. subtilis*, *B. licheniformis* and *B. pumilus* (Salkinoja-Salonen et al, 1999, From et al, 2005) and that virtually all were haemolytic. Consequently, the FEEDAP Panel concluded that the exclusion of such strains would be adequate to ensure consumer safety without precluding the use of *Bacillus* species in animal nutrition. Accordingly, the guidance available to applicants recommended an initial test for haemolysis followed by PCR detection of non-ribosomal peptide synthetase genes if the strain proved non-haemolytic. A positive PCR reaction was taken as indicative of a capacity to synthesise surfactins.

3. New evidence

Contrary to the original view that surfactin-like cyclic peptides were produced only by a small sub-set of bacilli, in a recent study, 53 strains of *B. licheniformis* isolated from different sources were all found to produce lichenysin (Madslien et al., 2013). The amount of production varied by more than two orders of magnitude, and the amount produced by some strains could only be detected by LC-MS/MS. However, the regulatory mechanisms controlling lichenysin production are unknown, so the amount produced by each strain may vary under different conditions. Relatives from the *B. subtilis* group are expected to behave similarly, and indeed this was found in a study from airborne bacteria in a subway station in Norway (Dybwad et al., 2012). By analysing for the presence of the genes making the surfactin-like toxins and analysing the different strains by using LC-MS/MS the picture seems to be very different than in the earlier studies.

In a similar exercise (Cocconcelli, personal communication) an examination of the published genomes of *B. subtilis* and related species was performed to detect the presence of genes coding for non-ribosomal peptide synthetases. The analysis was performed on a total of 26 complete whole genome sequences and 35 draft genomes of the species, *B. amyloliquefaciens, B. atrophaeus, B. subtilis B. licheniformis, B. mojavensis, B. sonorensis* and *B. vallismortis* (http://www.ncbi.nlm.nih.gov/genome). This *in silico* approach demonstrated that all the *Bacillus* strains for which the genome is available harbour at least one operon coding for more than one non-ribosomal peptide synthetase.

In now appears that the synthetic apparatus for the production of surfactin-like cyclic peptides is universally present in B. subtilis and the related species which represent the large

majority of commercially important strains. The present position adopted by the Panel that any indication of a capacity to produce such compound represents a hazard and should be avoided now appears disproportionate to the risk posed. Consequently, the FEEDAP Panel proposes to revise its guidance.

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Annex III

Questions from Observers

What steps do EFSA take to ensure coherence in guidance across EFSA panels? [Elinor McCartney (Pen & Tec Consulting SL)]

When new guidance documents are developed or updated, the Panel makes sure that all applicable or relevant guidance documents are considered where appropriate. For instance, when the FEEDAP Panel last year updated most of the guidance documents for the assessment of feed additives, recent guidance documents adopted by the Scientific Committee were taken into consideration some. The Scientific Committee has a key role in harmonising risk assessment approaches across EFSA.

Moreover, internal consultation procedures are in place to make sure that cross-cutting issues are considered. However, it should also be realised that different regulatory frameworks apply to the different areas of work of the Panels.

To what extend does the FEEDAP Panel take into consideration scientific guidance proposed by international organisations (e.g., VICH) when developing guidance on feed additives and what about evaluations performed by other international scientific bodies? [Davy Van Gaver (Huvepharma)]

When drafting the different guidance documents, an extensive search of the available guidance documents and practices from other national or international bodies (e.g., EMA, JECFA, VICH, FDA, OECD) is done. Whenever possible, harmonisation with existing guidance documents is sought. Similarly, during the assessment of feed additives, one of the aspects considered is whether a previous evaluation by other national or international bodies exists. If such an assessment is available, it is considered in conjunction with the data submitted by the applicant.

Are FEEDAP Members aware of the Fediaf Nutritional Guidelines developed by industry in cooperation with a Scientific Advisory Board composed of independent scientists? [Thomas Meyer (FEDIAF)]

Yes, the FEEDAP Panel is aware of the FEDIAF Nutritional guidelines for complete and complementary pet food for cats and dogs, and has consulted it on occasion.

The European Partnership for Alternative Approaches to Animal Testing (EPAA), a joint initiative from the European Commission and industry, was launched for the purpose of promoting the development, validation and acceptance of alternative approaches to further the replacement, reduction and refinement (3Rs) of animal use in regulatory testing.

Is the FEEDAP Panel taking into account the outcome of EPAA's activities when reviewing the Guidance documents for (re-)authorisation of feed additives? [Giuseppe Simone (FEDIAF)]

EFSA and the FEEDAP Panel in particular is very sensitive to the use of animals in experiments. The Panel fully supports the 3Rs approach and does not require studies in animals if they are not considered indispensable. Indeed, the Panel encourages use of alternative methods. As examples we can cite the guidance on user safety stating "the use of *in vitro* methods whenever possible" or the statement in the guidance on consumer safety "Where appropriate, validated alternative methods reducing the use of animals can be used". Also the Panel is concerned about animal welfare. For instance, in the guidance on tolerance and efficacy, we recommend that "… trials using a severely deficient control groups should be avoided".

Another example of the Panel's effort to reduce animal testing outside the area of laboratory animals is all the extrapolation of data obtained in major species to minor species.

The guidance on the renewal of authorisation does not contain specific requirements for toxicological testing or animal experimentation. The only animal studies which are specifically requested are sensitivity studies for coccidiostats, which are not easily replaceable with other alternative methods.

Are there (and which) animal tests currently required for feed additives authorisation that could be avoided by implementing the approaches suggested by EPAA? [Giuseppe Simone (FEDIAF)]

The Panel does not propose for the time being any alternative methods. However, if these methods are available and properly validated, the Panel will evaluate their suitability during the assessment of the technical dossiers.

The FEEDAP Panel have tried to consider results from alternative studies, such as *in vitro*, *in sacco* and/or *in silico* studies. To be taken into account it is important that they have been be validated in animal studies. Other techniques (e.g. -omics techniques) are likely to become important in the future. As an example of FEEDAP's dedication to reduce the number of animals I can mention that acute toxicity is not required anymore in the new Guidance of Consumer safety.

However, in the attempt to reduce the number of animals it is important to make sure that the necessary statistical power of the experiments is maintained. The ramifications of erroneous decisions obviously can be great.

As a consequence of the EPAA's activities and of the 3Rs does the FEEDAP Panel hold the opinion that a revision of Regulation 1831/2003 and of Regulation 429/2008 is required? [Giuseppe Simone (FEDIAF)]

Regulation (EC) No 429/2008, as well as the Panel's guidance documents already encourages the use of alternative methods: "The use of *in vitro* methods or of methods refining or replacing the usual tests using laboratory animals or reducing the number of animals used in these test shall be encouraged. Such methods shall (…) provide the same level of assurance as the method they aim to replace." Anyway, the decision on the need to modify the regulations above mentioned rests in the European Commission.

In relation to the renewal of coccidiostat authorisations, why do EFSA consider that efficacy studies should be updated, when this is not the case for other categories of additives? [Elinor McCartney (Pen & Tec Consulting SL)] (Guido Rychen)

Development of resistance of *Eimeria* to coccidiostats is quite widespread and can occur rapidly. In the case of coccidiostats, lack of efficacy may lead to safety problems. Therefore, it is important to ensure that the additive is still efficacious under the proposed conditions of use. It should be noted that the efficacy requirements are limited to sensitivity studies and that it is not required to produce a full set of studies as it would be the case for a new authorisation of a coccidiostat.

FEFANA is acknowledging that Regulation 429/2008 does not give details on requirements for the technological feed additives "mycotoxin binders and inactivators". Therefore, a common understanding on how such additives should be assessed is needed. Would the FEEDAP Panel agree to re-discuss the current requirements set in the EFSA guidance for technological feed additives with e.g. FEFANA? Gerald Schultheis (FEFANA Working group Mycotoxins)

EFSA was requested in 2009 by the European Commission to provide technical advice on the guidelines to be followed for the submission of dossiers for applications for authorisation of additives belonging to the functional group of substances for reduction of the contamination of feed by mycotoxins. At that time the FEEDAP Panel made a proposal for the requirements to be followed and held a meeting with stakeholders on 8 June 2010. During that meeting the views of several representatives from individual companies and from associations like FEFANA were presented and discussed. All comments received prior and during the meeting were considered when the final version of the proposed guidance was adopted. At present, the FEEDAP Panel does not consider that there is a need to revise the guidance regarding substances for reduction of the contamination of feed by mycotoxins.

Does the update of the guidance on the assessment of the toxigenic potential of *Bacillus* affect current QPS status of *Bacillus* species? [Noriko Nakamura (Calpis Co. Ltd.)]

No it doesn't. The qualification in the QPS document for *Bacillus* species is absence of toxigenic potential. No information is given in the QPS opinion on how the absence of toxigenicity should be demonstrated, which is left to specific guidance documents. The FEEDAP Technical Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition is a standalone document (which complements the QPS opinion) aimed at providing applicants with proportionate and up-to-date guidance on how to conduct the safety assessment of *Bacillus*-based products. Its update, however, does not affect in any way the principles/provisions established in the QPS approach to safety assessment.

How do EFSA reconcile that "toxins" in one genome, may be considered "niche factors" in another, e.g. adherence genes? [Elinor McCartney (Pen & Tec Consulting SL)]

There is a difference between toxins and putative virulence factors. Toxins are poisonous substances produced by microbial cells. For most of the toxins (e.g. *Bacillus* cereus enterotoxins or cereulide) the mode of action is known and there is a clear linkage between the exposure and the disease. For other putative virulence factors, such as adherence genes, in most of cases there is only indirect demonstration of their role in pathogenesis. In this case the assessment is made taking in consideration the complete context (the bacterial species, the gene, the available data, etc.)

Bacillus subtilis **and certain other** ***Bacillus*** **species have QPS status and have proven through 50 years of industrial use to be very safe organisms to use as hosts for production of enzymes. The new strict guidelines on lipopeptides seem to be in strong contrast to the history of safe use of** ***Bacillus*** **and will jeopardise all currently approved** ***Bacillus*** **production strains and strains used as food and feed probiotics since all known bacilli have the genetic capacity to produce lipopeptides. Does the FEEDAP Panel agree to the above statements? [Michael Dolberg Rasmussen (Novozymes)]**

The Panel acknowledges that for some additives, including microorganisms, there is a history of use that might support their safety evaluation. However, the main objective of the re-evaluation exercise is to have all feed additives assessed under the same scientific criteria.

Early data suggested that the surfactin-like cyclic peptides production in the *Bacillus subtilis* and related species was limited to 3-4% of the strains. However, recent studies on the genomics of *Bacillus* and involving different strains in which genes of making the surfactin-like toxins have been analysed suggest that the picture is very different. In view of this new evidence, the FEEDAP Panel has decided to update the guidance.

The data used by the Panel to substantiate the health issue for lipopeptides has recently been refuted by several external ***Bacillus*** **experts. It seems in particular that the recommendations leading to these guidelines were based on inadequate data and conclusions. FEEDAP is indeed in the process of revisiting the guidelines. Are there any indications of where this amendment process is headed, and what are the basic assumptions behind it? [Michael Dolberg Rasmussen (Novozymes)]**

The FEEDAP Guidance documents are “living” documents that need to be updated according to the latest scientific and technical developments. Since the Technical Guidance on the assessment of the toxigenic potential of *Bacillus* species was issued, several publications on the toxicity and prevalence of non-*Bacillus* cereus toxins have become available. Therefore, the FEEDAP Panel in view of this and of the experience gained so far from the assessment of the toxigenic potential of products based on *Bacillus* species has updated this document. According to EFSA’s principles of transparency, the draft updated Guidance will be subject to public consultation. Final adoption will take place only upon consideration of the comments received from stakeholders.

The industry is strongly concerned with a potential trend from EFSA to use genome data interpretations as a sole reason to reject strains (as live microorganisms or

production strains for e.g. enzymes). Would EFSA agree to organise a stakeholders' meeting on this topic? [Michael Dolberg Rasmussen (Novozymes)]

The safety assessment of bacterial strains used as feed additives is based on a number of factors, and the genome sequence is just one of them. Genomic data provide a deep view inside the potential metabolism of a microbial cell and can highlight potential hazards. For instance, the presence of genes coding for transferable antibiotic resistance genes in strain intentionally introduced in the food chains as viable feed additive is *per se* considered a risk.

Molecular typing or microbial DNA fingerprinting has developed rapidly in recent years. Many typing methods, like PCR techniques and sequencing, have become part of routine strain characterisation in many laboratories. Molecular typing provides essential tools for the early detection and thus, potential prevention of outbreaks. In fact, EFSA, ECDC and the EC (EURLs) have signed an agreement to closely collaborate on molecular typing of foodborne pathogens, and to take the responsibility for the molecular typing data collection as regards food and animal isolates.

At present EFSA is not considering organising a stakeholders meeting regarding this topic, but it may do in the future.

Max limit for Selenium applies to all species. Under which circumstances would the panel endorse a pet (cats & dogs) specific regulation? [Thomas Brenten (Mars GmbH)]

The maximally allowed total concentration of selenium in animal feeds - and this applies to all animal species - is 0.5 mg Se/kg feed. This limit covers the requirements and allowances for cats and dogs of 0.25 – 0.3 mg Se/kg feed as published by NRC (NRC, 2006). It also serves to protect target animals against accidental overdose caused for example by variability in Se content of feed materials or non-homogenous mixing of the additive. Finally, the maximal Se content in all feeds for food-producing animals is intended to protect consumers from Se excess although this is hopefully not of relevance for cat and dog feeds.

EFSA's principal mission is to protect the consumer, the user, the target animals and the environment from unsafe food, feed and practices involved in food production. There are two signals to which FEEDAP will react. It will respond to questions asked by the Commission and it will re-assess additives and their concentrations in feed should there be evidence that there is a potential risk. As an example of the former, the FEEDAP Panel assessed, on a request from the Commission, the potential impact of reducing vitamin A concentrations in animal feeds and concluded that some reductions could be made without jeopardising the health of farm animals. (Another example is the opinion on iodine). As an example of the latter, FEEDAP recognised the higher deposition of Se in tissues and products from the use of feed additives based on selenomethionine (SeMet), compared to inorganic forms, and recommended a proportional reduction in the maximum organic Se content (to 0.2 mg organic Se/kg) to protect consumers from potential Se toxicity. The Commission recently approved this change for all animal species although the risk would not apply to non food-producing animals, such as pets.

The application dossiers for the re-evaluation of feed additives have been prepared before November 2012. In the meantime there might have occurred changes in

product specifications, analytical methods or scientific literature which are relevant for the dossier. Is there any procedure for updating an application dossier before or during the evaluation by EFSA? [Renato Rosà (EMFEMA)]

In principle, the dossier when submitted to EFSA should include the most up to date information. If the Panel required additional information, the applicant will be requested. Depending on the new information that is available the way to proceed may be different. If there is a significant change in the product specifications, the additive might be a new one and therefore, a new application with a new technical dossier will be required. If it is new supporting evidence, this might be submitted during the course of the evaluation. It is preferable that any new information is submitted before the assessment has started, i.e., during the "missing parts" process. Data submitted at a later stage may not be considered by the Panel.

How can an applicant of a feed additive registration react on a Scientific Opinion on its product in case the opinion contains errors (according to the applicant)? [Stefan Wittocx (Orffa Belgium NV)]

After an opinion has been adopted, the applicant is immediately notified and receives a copy of the adopted opinion. The opinion at that stage is subject to proof-reading before it is published on the website, which will normally happen within 2 weeks of its adoption. During this proof-reading most editorial errors will be corrected. The applicant is also pre-notified 24 h before the opinion is published on the website. If the applicant realises that the opinion contains errors, he/she can directly notify the FEED unit by e-mail indicating the nature of the error. The FEED Unit will then take the actions it considers opportune to correct the error. Errors of editorial nature are corrected right away. Applicants do not always agree to the conclusions that the Panel has reached and asks the Panel to change either the conclusions or the wording. However, this is normally not considered an error. In the exceptional cases in which an error of scientific nature is identified, there are mechanisms in EFSA to review the output and proceed with corrective actions.

Is it possible to have a dialogue (a real 2-way communication) between the applicant and EFSA, right before or after the adoption of the scientific opinion, especially about the recommendations EFSA makes? [Stefan Wittocx (Orffa Belgium NV)]

At present, it is not foreseen that the opinion is made available to the applicant before adoption. Once the opinion is adopted, it is submitted to the applicant. However, it is not possible to change the contents of the opinion after it has been adopted.

I would like to discuss if, in view of more transparency, a system for oral hearings can be set up. [Bert Soenen (Elanco)]

It is EFSA's policy not to hold technical hearings with individual applicants. EFSA is envisaging to organise some meetings with applicants on exceptional cases (e.g, post adoption of the opinion). However, EFSA is keen in providing as much support as possible to applicants before the submission of the applications and during the assessment either via the dedicated Apdesk Unit or the FEED Unit. Direct contacts between industry and experts are not allowed.

Is it possible to discuss scientific issues with the workgroup or FEEDAP Panel? This may make it more efficient (as for veterinary medicines) to explore solutions for problems that may be encountered during the registration process. [Davy Van Gaver (Huvepharma)]

It is EFSA's policy not to hold technical hearings with individual applicants. EFSA is envisaging to organise some meetings with applicants on exceptional cases (e.g, post adoption of the opinion). However, EFSA is keen in providing as much support as possible to applicants before the submission of the applications and during the assessment either via the dedicated Apdesk Unit or the FEED Unit. Direct contacts between industry and experts are not allowed. It is expected that the technical dossier contains all the information necessary to reach a conclusion regarding the safety and the efficacy of the additive. Experience has proved that a careful preparation of the technical dossier before submission and of the supplementary information, where relevant, is the best way to address the concerns that the Panel might have during the evaluation. It should also be borne in mind that the evaluation of medicines and additives follow different regulations and procedures, and while for veterinary medicines there are fees associated with the process, this is not the case for feed additives.

The scientific opinion is a valuable document, however it would be useful for the applicant to receive a more thorough evaluation and annotated document (applicant only) in which decisions can be motivated and/or points of attention can be highlighted for a future renewal. Is this possible? [Davy Van Gaver (Huvepharma)]

The scientific opinion contains all information that the FEEDAP Panel has used to reach a conclusion on the safety/efficacy of a given feed additive. The assessment of a feed additive is done considering the legal requirements established in Regulation (EC) No 429/2008 and the applicable guidance documents. The opinion follows the structure and requirements established in these documents and, in principle, contains all reasoning behind the conclusions and highlights where deficiencies (if any) are. The FEEDAP Panel is making efforts to ensure that its opinions are complete and understandable.