SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 19 October 2016

doi: 10.2903/j.efsa.2016.4620

Safety and efficacy of Feedlyve AGL (endo-1,3(4)-β-glucanase) as a feed additive for chickens for fattening

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Abstract

Feedlyve AGL is a feed additive that is available in liquid and solid formulations and contains endo-1,3(4)-β-glucanase, which is produced by a strain of *Aspergillus niger*. The tolerance trial submitted did not comply with the requirements of tolerance trials, and consequently, the study was considered not valid. Therefore, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) could not conclude on the safety of the additive for the target species. The enzyme concentrate gave negative results in a bacterial reverse mutation assay and in an *in vitro* chromosome aberration assay. Moreover, the substance was negative in an *in vivo* micronucleus test in the rat bone marrow and the results of the subchronic oral toxicity study showed no adverse effects. However, the correspondence of the test item used and the fermentation product that is currently used to prepare the additive was not established. Therefore, the FEEDAP Panel was not in a position to conclude on the safety for the consumer of the additive. The tests conducted in order to address the safety for the user indicated that the test items were not toxic by inhalation, not irritant to the eyes or skin but showed a dermal sensitisation potential. However, the relationship between the test items and the additive for which re-evaluation is sought were not fully established. The additive is considered as a potential respiratory sensitizer. No risks to the environment are expected. The results of two efficacy trials showed that 100 AGL U/kg feed increased the apparent metabolisable energy of the diets in one trial and improved the body weight gain and the gain to feed ratio in another trial. However, a third trial showing significant and positive effects would be required to positively conclude on the efficacy of the product.

Keywords: zootechnical additives, digestibility enhancers, safety, efficacy, glucanase, chickens

Requestor: European Commission

Question number: EFSA-Q-2014-00229

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Acknowledgements: The Panel wishes to thank Jaime Aguilera for the support provided to this scientific opinion.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López Puente S, López-Alonso M, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Brantom P, Dierick N and Anguita M, 2016. Scientific opinion on the safety and efficacy of Feedlyve AGL (endo-1,3(4)-β-glucanase) as a feed additive for chickens for fattening. EFSA Journal 2016;14(11):4620, 11 pp. doi:10.2903/j.efsa.2016.4620

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Feedlyve AGL as a feed additive for chickens for fattening. This additive is authorised for use as a feed additive in chickens for fattening and the applicant requested for the re-evaluation of the product. Feedlyve AGL is a preparation of endo-1,3(4)-β-glucanase (glucanase) that is available in liquid and solid formulations. The enzyme that is contained in the additive is produced by a strain of *Aspergillus niger*. However, the identity of the strain was not confirmed by molecular techniques.

One tolerance trial in chickens for fattening was evaluated. The animals were under study from day 7 to day 21 of life. This duration was not in accordance with the requirements for tolerance trials in terms of age at start and duration of the treatment (start on day 1 of life and study for 35 days). Moreover, the study lacked replicates, and therefore, the statistical evaluation of the data would not be possible. The Panel considered this study as not valid and could not conclude on the safety of the additive for the target species.

The enzyme concentrate gave negative results in a bacterial reverse mutation assay and in an *in vitro* chromosome aberration assay. Moreover, the substance was negative in an *in vivo* micronucleus test in the rat bone marrow, although no evidence of target cell exposure was provided. The results of a subchronic oral toxicity study showed no adverse effects. These tests were conducted in the 1990s, with the exception of the *in vivo* micronucleus test, which was performed in 2010. The correspondence of the test item with the fermentation product that is currently used to formulate the additive was not established. Therefore, the FEEDAP Panel was not in a position to conclude on the safety for the consumer of the additive.

An acute inhalation study, the skin and eye irritation tests and a skin sensitisation study were performed and indicated that the test items were not toxic by inhalation and not irritant to the eyes or skin but had a dermal sensitisation potential. However, the relationship between the test items and the additive for which re-evaluation is sought were not fully established. Owing to the proteinaceous nature of the active substance, the additive is considered as a potential respiratory sensitiser.

The active substance of the additive is a protein and as such will be degraded/inactivated during the passage through the digestive tract of animals. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

Five efficacy trials, three short-term and two long-term, were submitted. Two of the short-term trials were not considered further due to the nature of the parameters measured. One of the long-term trials was not considered further due to several limitations in the methodologies followed and reporting that were not addressed/explained by the applicant. The results of the other two trials showed that the addition of 100 AGL U/kg feed to the diets increased the apparent metabolisable energy of the diets in one trial and improved the body weight gain and the gain to feed ratio in another trial. However, another trial showing significant and positive effects would be required to positively conclude on the efficacy of the product.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from LYVEN[2] for re-evaluation of the authorisation of the product Feedlyve AGL (endo-1,3(4)-β-glucanase), when used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: digestibility enhancers).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 16 April 2015.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment, and on the efficacy of the product Feedlyve AGL (endo-1,3(4)-β-glucanase), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Feedlyve® AGL is a preparation of endo-1,3(4)-β-glucanase (EC 3.2.1.6) produced by a strain of *Aspergillus niger* (MUCL 39199). This product is authorised in the European Union (EU) as a feed additive for chickens for fattening.[3] The Scientific Committee on Animal Nutrition issued an opinion on the safety of use of this additive for the target species (chickens for fattening), consumers and users (EC, 2002).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of Feedlyve AGL as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[5] and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the Feedlyve AGL in animal feed. The Executive Summary of the EURL report can be found in Annex A.[6]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] LYVEN ZAC Normandial, 11 Avenue du Pays de Caen, 14460 Colombelles, France.
[3] Commission Regulation (EC) No 1458/2005 of 8 September 2005 concerning the permanent and provisional authorisations of certain additives in feedingstuffs and the provisional authorisation of new uses of certain additives already authorised in feedingstuffs. OJ L 233, 9.9.2005, p. 3–7.
[4] FEED dossier reference: FAD-2010-0227.
[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2010-0227-feedlyve_agl.pdf

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Feedlyve AGL is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

This opinion deals with the re-evaluation of the safety and efficacy of FEEDLYVE AGL (endo-1,3(4)-β-glucanase, (EC 3.2.1.6; glucanase)) as a feed additive for chickens for fattening.

3.1. Characterisation of the active substance

The main activity of the additive is glucanase but other side activities are present (non-starch polysaccharidases). The glucanase is obtained by fermentation with a strain of *Aspergillus niger* deposited in 1995 at the Mycothèque de l'Université Catholique de Louvain with the accession number (MUCL 39199). No evidence was provided that the strain is still deposited in the culture collection.[7] The identification of the strain was carried out by morphological examination,[8] but no confirmation of the identity was provided using molecular techniques. Characteristics of the growth of the strain and ability to produce glucanase were provided along with the genomic fingerprinting of the strain.[9] However, the identification was not confirmed using molecular techniques. This strain is a mutant as stated in the certificate of deposition, but no details on the methodologies and steps followed to select the strain were provided in order to support the statement. The genetic stability of the strain was not studied and no data on the potential of the production strain to produce potentially toxic secondary metabolites was provided.

3.1.1. Manufacturing process[10]

The enzyme is obtained by a multi-step process consisting of fermentation, concentration and purification steps. The product obtained is stabilised. The liquid formulations are obtained from this product by addition of the carriers/stabilisers. For the preparation of the solid formulations, the stabilised liquid is spray-dried. The product obtained is used to prepare the solid formulations (referred to as Feedlyve AGL BRUTE (CNS)). Indications on the enzyme activity at the different points of the manufacturing were provided, expressed per initial dry matter in the fermenter.

The dossier contains information on the spray-dried product used to prepare the solid formulations, Feedlyve AGL BRUTE (CNS). The batch-to-batch variation in the enzyme activity in five batches showed mean values of 12,627 AGL U/g (range: 12,012–14,030).[11,12]

3.1.2. Characterisation of the additive

The additive is available in two solid (Feedlyve AGL 1500 P and 6000 P) and two liquid forms (Feedlyve AGL 200 L and 1500 L). Another formulation, Feedlyve AGL 400 P, was mentioned in the dossier but the information provided does not allow a full characterisation.

Feedlyve AGL 1500 P and 6000 P are solid formulations standardised to ensure 1,500 or 6,000 AGL U/g, respectively. The study of the batch-to-batch variation in five batches showed a mean value of 1,777 AGL U/g (range 1,560–2,154, with a coefficient of variation (CV) of 14%) for Feedlyve AGL 1500 P and a mean value of 6,912 AGL U/g (range 6,345–8,000; CV of 10%) for Feedlyve AGL 6000 P.[13] These formulations contain Feedlyve AGL BRUTE (CNS), 0.5% tricalcium phosphate and

[7] Technical dossier/Section II/Annex II.2.1.B.
[8] Technical dossier/Section II/Annex II.2.1.C and II.2.1.D.
[9] Technical dossier/Section II/Annex II.2.1.E–2.1.G and Annex II.2.1.Fbis.
[10] This section has been amended following the confidentiality claims made by the applicant.
[11] AGL U is the amount of enzyme which liberates 5.55 μmol of reducing sugars (glucose equivalents) from barley β-glucan per minute at pH 4.8 and 30°C.
[12] Technical dossier/Section II/Annex II.1.3.B.
[13] Technical dossier/Section II/Annex II.1.3.A.

wheat flour. Data on the physicochemical properties of the two solid formulations were submitted, including pH, density, particle size distribution and dusting potential.[14] The study of the particle size (by sieving) in three batches of each formulation was provided and the particles < 40 μm ranged from 53 to 58% for Feedlyve AGL 1500 P and 34–68% for Feedlyve AGL 6000 P, but the data did not include information on particle size distribution below 40 μm. The dusting potential was measured in one batch for each formulation and was 0.1 mg/50 g for the 1500 P formulation and 2.3 mg/50 g product for the 6000 P.

Feedlyve AGL 200 L and 1500 L are the liquid formulations standardised to ensure 200 or 1500 AGL U/g, respectively. The study of the batch-to-batch variation in three batches showed a mean value of 245 AGL U/g (range: 230–262) for Feedlyve AGL 200 L and a mean value in five batches of 1,612 AGL U/g (range: 1,516–1,742; CV of 6%) for Feedlyve AGL 1500 L.[15] These formulations contain the liquid product obtained after fermentation, 30% sorbitol, 5% monopotassium phosphate, 0.02% potassium sorbate and water.[16] The pH of these formulations is 5 and the density is 1.16–1.18 kg/m^3.[17]

The purity of the final formulations was not provided; however, data on one batch of the fermentation product (liquid) was made available for chemical and microbial purity.[18] Chemical analysis included lead (0.06 mg/kg), arsenic (< 0.10 mg/kg), cadmium (< 0.05 mg/kg) and mercury (< 0.03 mg/kg). Mycotoxins including aflatoxins (< 0.1 μg/kg), zearalenone (6.0 μg/kg) and ochratoxin (0.5 μg/kg) were also analysed in these batches. Microbial analysis was done for total viable counts (< 1,000 colony-forming units (CFU)/g), *Salmonella* spp. (absent in 25 g), coliforms, *Escherichia coli* (< 1 CFU/g), anaerobic sulfate reducers (< 10 CFU/g) and *Staphylococcus aureus* (absent in 1 g). The test on the presence of antibiotic activity revealed no evidence of such activity. The same information (including also the absence of the production strain) was provided for three batches of Feedlyve AGL BRUTE (CNS),[19] batches from 1993 to 1995, but the correspondence of the batches analysed with the product that is currently used to manufacture the additive has not been established.

3.1.3. Stability and homogeneity

3.1.3.1. Shelf life

Three batches of the liquid (200 L and 1,500 L) and solid formulations (1500 P and 6000 P) were kept at three different temperatures (4, 20 and 30°C) in closed packages for 3 months (liquid) or for 12 months (solid).[20] The enzyme recoveries (in %) were calculated as the activity present in the test sample compared to the activity present in a frozen sample at the same time point. Recoveries were > 95% after 3 months in all situations studied. The test also included the study of the effect of light (by comparing samples in darkness and the effect of open or closed packages), air and humidity, this last test was done only in the solid formulations.[21] These factors had no impact on the shelf life of the enzyme.

The data provided showed the following limitations: the claimed shelf life (2 years) was not studied in any of the tests, the initial enzyme activity of the batches was not provided and the recoveries were calculated comparing the enzyme activity to a frozen sample, which could have also lost activity.

3.1.3.2. Stability and homogeneity of the additive

The stability of three batches of the two solid formulations (1500 P and 6000 P) added to a vitamin/mineral premixture (without choline chloride), enzyme activity of 25 U/g, was studied. Samples of the premixture were stored at 4, 20 and 30°C for up to 4 months.[22] After 4 months, mean enzyme activity was > 95% in all cases (expressed as compared to a frozen sample). No data were provided on the stability in premixtures up to 6 months of storage.

The stability of the additive (unknown formulation) was studied when added to a complete compound feed and then pelleted at 75, 78 or 80°C.[23] Recoveries were < 33%, indicating low stability of the enzyme to heat treatment. The stability of three batches of the liquid (200 L and 1,500 L) and

[14] Technical dossier/Section II/Annexes II.1.5.B and II.1.5.C.
[15] Technical dossier/Section II/Annex II.1.3.A and II.1.5.B.
[16] Technical dossier/Section II/Annex II.1.3.
[17] Technical dossier/Section II/Annex II.1.5.B.
[18] Technical dossier/Section II/Annex II.2.1.J.
[19] Technical dossier/Section II/Annex II.1.4.A–1.4.E.
[20] Technical dossier/Section II/Annex II.4.1.A.
[21] Technical dossier/Section II/Annex II.4.1.B and II.4.1.C and Annex II.4.1.D.
[22] Technical dossier/Section II/Annex II.4.1.E and Annex II.4.1.J.
[23] Technical dossier/Section II/Annex II.4.1.H and Annex II.4.1.F.

solid formulations (1500 P and 6000 P) added to a complete feed (mash form for solid and pelleted for liquid) at an enzyme activity of 100 AGL U/kg feed was studied. Samples of the feed were stored at 4, 20 and 30°C for up to 3 months.[24] Mean recovery values were > 94% (expressed as compared to a frozen sample).

The premixture and mash feed used for the stability studies were sampled five times (less than the required 10 subsamples) in order to study the capacity of the additive to distribute homogeneously.[25] Samples in premixtures showed a CV < 6%, and samples in feed showed CV of 6% for Feedlyve AGL 1500 P and < 12% for the Feedlyve AGL 200 L.

3.1.4. Conditions of use

The additive is to be used in chickens for fattening at a minimum dose of 25 AGL U/kg feed and a maximum dose of 100 AGL U/kg feed up to 35 days.

3.2. Safety

3.2.1. Safety for the target species

The applicant submitted one trial in chickens for fattening[26] to support the safety for the target species.[26] However, this study is considered not acceptable since the animals were under study from day 7 to day 21 of life and there were no replicates. Therefore, the Panel cannot conclude on the safety of the additive for the target species.

3.2.2. Safety for the consumer

The applicant submitted a bacterial reverse mutation assay (OECD TG 471),[27] an *in vitro* chromosome aberration assay (OECD TG 473),[28] an *in vivo* micronucleus test (OECD TG 474)[29] and a subchronic oral toxicity study (OECD TG 408).[30]

The test item used for these studies was Feedlyve AGL BRUTE (CNS) which was claimed to be the non-standardised spray-dried fermentation product with dextrin and monopotassium phosphate used in the formulation of the solid formulations. The tests items used in these tests were manufactured in the 1990s with the exception of the one used in the *in vivo* micronucleus test, that was from 2010. The correspondence of those test items and the fermentation product that is currently used to formulate the additive was not established.

The results obtained in the studies submitted do not indicate any reason for concern for consumer safety arising from the use of the additive as a feed additive. However, since the correspondence of the test item and the fermentation product that is currently used has not been established, the FEEDAP Panel is not in a position to conclude on the safety for the consumer of the additive.

3.2.3. Safety for the user

An acute inhalation study (OECD TG 403),[31] the skin (OECD TG 404) and eye (OECD TG 405) irritation studies[32] and a skin sensitisation study (OECD TG 406)[33] were submitted. Feedlyve AGL BRUTE (CNS) was the test item in the acute inhalation study and in the skin sensitisation study, and a test item identified as 'Glucanase 6400' was used in the skin and eye irritation studies.

The test materials were not toxic by inhalation and not irritant to the skin and eyes but showed a moderate dermal sensitising potential. However, their correspondence with the product for which re-evaluation is sought was not established; therefore, the FEEDAP Panel is not in the position to conclude on these properties. Owing to the proteinaceous nature of the active substance, the additive is considered as a potential respiratory sensitiser.

[24] Technical dossier/Section II/Annex II.4.1.K and Annex II.4.1.F.
[25] Technical dossier/Section II/Annexes II.4.2.A–4.2.C.
[26] Technical dossier/Section III/Annex III.1.A.
[27] Technical dossier/Section III/Annex III.2.2.B.
[28] Technical dossier/Section III/Annex III.2.2.C.
[29] Technical dossier/Section III/Annex III.2.2.H.
[30] Technical dossier/Section III/Annex III.2.2.D–2.2.G.
[31] Technical dossier/Section III/Annex III.2.2.A.
[32] Technical dossier/Section III/Annex III.3.1.B and Annex III.3.1.A.
[33] Technical dossier/Section III/Annex III.3.1.C.

3.2.4. Safety for the environment

The active substance of the additive is a protein and as such will be degraded/inactivated during the passage through the digestive tract of animals. Therefore, no risks to the environment are expected and no further environmental risk assessment is required.

3.3. Efficacy for chickens for fattening

Five trials, three short-term and two long-term, were submitted. Two of the short-term trials were not considered further due to the nature of the parameters measured.[34] One of the long-term trials was not considered further due to several limitations in the methodologies followed[35] and reporting[36] that were not addressed/explained by the applicant.

In the short-term trial considered,[37] a total of thirty-six 14-day-old male chickens for fattening were distributed in groups of three birds to two dietary treatments (representing six replicates per treatment). A basal diet based on barley and soya bean meal was either not supplemented (control) or supplemented with Feedlyve AGL 1500 P to provide 100 AGL U/kg feed. The enzyme activity was confirmed by analysis. The diets were offered to the birds for 7 days in mash form and contained titanium dioxide as an external marker. On day 21 of life, samples of the excreta were collected and analysed for gross energy, nitrogen and the marker. The apparent metabolisable energy corrected for nitrogen of the diets was calculated. An analysis of variance was performed with the data and the comparison of the mean values of the two treatments was done with a t-test; the statistical significance level was set at $p < 0.05$. Results showed that the apparent metabolisable energy (nitrogen corrected) contained in the diets supplemented with the enzyme was significantly higher than in the non-supplemented one (from 10.54 to 11.63 MJ/kg feed).

In the long-term trial,[38] a total of 360 one-day-old male chickens for fattening (Cobb) were grouped in pens of 30 birds and allocated to two dietary treatments (representing six replicates per treatment). Basal diets (starter and grower) based on barley and soy were either not supplemented (control) or supplemented with Feedlyve AGL 1500 P in order to provide 100 AGL U/kg feed. The enzyme activity was confirmed by analysis. Feed was offered on *ad libitum* basis and in mash form to the birds. Health status of the birds and mortality were checked throughout the study. Birds were weighed and feed intake was measured in a pen basis. Feed to gain ratio was calculated. An analysis of variance was performed with the data; the statistical significance level was set at $p < 0.05$. Seven birds died during the experiment, four from the control diet and three from the 100 AGL U/kg feed diet. No differences were found between the dietary treatments on the feed intake (4.3 kg per bird). Compared to the control, the supplemented diet significantly increased the body weight gain of the birds (2.0 vs 2.2 kg) and improved the gain to feed ratio (0.47 vs 0.52).

The dose of 25 AGL U/kg feed was not tested in any of the two trials considered. The results showed that the addition of 100 AGL U/kg feed (maximum dose) to the diets permitted to have a higher apparent metabolisable energy of the diets in one trial and a higher body weight gain and better gain to feed ratio in another trial. However, another trial showing significant and positive effects would be required to positively conclude on the efficacy of the product.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[39] and Good Manufacturing Practice.

4. Conclusions

Owing to the lack of data/information, the FEEDAP Panel is not in the position to conclude on the identity of the production strain, the safety of the additive for the target species, consumer or on the

[34] Technical dossier/Section IV/Annex IV.2.B and IV.2.C.
[35] Including redistribution of birds after day 12 under study due to a high mortality registered after flooding.
[36] Discrepancies found in the information regarding the animals and their distribution, data reported per period but not in the overall, missing data regarding the distribution of the birds after flooding and analytical results of the enzyme activity missing.
[37] Technical dossier/Section IV/Annex IV.2.A.
[38] Technical dossier/Section IV/Annex IV.3.A.
[39] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

dermal/eye irritancy potential and the dermal sensitisation properties of the additive. The additive is to be considered a potential respiratory sensitiser. The use of Feedlyve AGL as a feed additive poses no risks to the environment.

The Panel cannot conclude on the efficacy of Feedlyve AGL as a feed additive for chickens for fattening.

Documentation provided to EFSA

1) Feedlyve AGL for chickens for fattening. November 2010. Submitted by LYVEN.
2) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Feedlyve AGL.
3) Comments from Member States.

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EC (European Commission), 2002. Opinion of the Scientific Committee on Animal Nutrition on the use of certain enzymes in animal feedingstuffs. Adopted on 4 June 1998, updated 16 October 2002. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed_additives_rules_scan-old_report_out96.pdf

EFSA (European Food Safety Authority), 2008. Technical Guidance of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) for assessing the safety of feed additives for the environment. EFSA Journal 2008;6(10):842, 28 pp. doi:10.2903/j.efsa.2008.842

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. doi:10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539

Abbreviations

CFU	colony-forming unit
CV	coefficient of variation
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
MUCL	Mycothèque de l'Université Catholique de Louvain
OECD	Organisation for Economic Co-operation and Development
TG	Technical Guideline

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Feedlyve AGL

Feedlyve® AGL is currently authorised as feed additive for chickens for fattening by Commission Regulation (EC) No 1458/2005. In the current application authorisation is sought under article 10 (2) of Regulation (EC) No 1831/2003 under the category/functional group "zootechnical additives"/ "digestibility enhancers" for chickens for fattening.

According to the Applicant, Feedlyve® AGL contains endo-1,3(4)-β-glucanase as active agent. The Applicant expresses the glucanase enzymatic activity in glucanase units (AGL), defined as "1 AGL, the amount of enzyme which liberates 5.55 μmol of reducing sugars (glucose equivalents) from barley beta-glucan per minute at pH 4.8 and 30°C". The product is intended to be marketed as solid and liquid formulations having a guaranteed minimum glucanase activity ranging from 200 to 6000 AGL/g of product. The feed additive formulations are intended to be included through premixtures or directly in feedingstuffs to obtain a minimum activity of 25 AGL/kg feedingstuffs. However the Applicant proposed a recommended dose ranging from 25 to 100 AGL/kg feedingstuffs.

For the quantification of the glucanase activity in the feed additive and feedingstuffs the Applicant submitted a single-laboratory validated and further verified colorimetric method based on the enzymatic hydrolysis of barley beta-glucan at pH 4.8 and 30°C, and the quantification of dyed oligomers released from the substrate Remazol-Brilliant-Blue-R glucan. Furthermore, the Applicant applied this method successfully for the quantification of glucanase in premixtures, in the frame of the stability studies. Based on the satisfactory performance characteristics available, the EURL recommends for official control of this colorimetric method for the quantification of the glucanase activity in the feed additive, premixtures and feedingstuffs.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.