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SCIENTIFIC OPINION

Safety and efficacy of a feed additive consisting of xylanase (produced with *Komagataella phaffii* DSM 25376) and β-glucanase (produced with *Komagataella phaffii* DSM 26469) (ENZY CARBOPLUS®) for all poultry (Kaesler Nutrition GmbH)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) | Roberto Edoardo Villa | Giovanna Azimonti | Eleftherios Bonos | Henrik Christensen | Mojca Durjava | Birgit Dusemund | Ronette Gehring | Boet Glandorf | Maryline Kouba | Marta López-Alonso | Francesca Marcon | Carlo Nebbia | Alena Pechová | Miguel Prieto-Maradona | Ilen Röhe | Katerina Theodoridou | Baltasar Mayo | Lieve Herman | Montserrat Anguita | Nicole Bozzi Cionci | Matteo L. Innocenti | Jordi Ortuño | Elisa Pettenati

Correspondence: feedap@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of xylanase (produced with *Komagataella phaffii* DSM 25376) and β-glucanase (produced with *Komagataella phaffii* DSM 26469) (ENZY CARBOPLUS®) as a zootechnical feed additive (functional group: digestibility enhancers). The additive is already authorised for use in feed for chickens for fattening, chickens reared for laying, turkeys for fattening and all avian species reared for laying or breeding purposes. The applicant requested a modification of the terms of the current authorisation for chickens for fattening and reared for laying, as well as an extension of use to all poultry. The FEEDAP Panel concluded that the additive is safe for all poultry. The use of the additive is considered safe for the consumers and the environment. The FEEDAP Panel concluded that the additive is not a skin or eye irritant nor a dermal sensitiser, but it is considered a respiratory sensitiser. The Panel concluded that the additive has the potential to be efficacious as a zootechnical additive for all poultry under the proposed conditions of use.

KEYWORDS

all poultry, digestibility enhancers, efficacy, ENZY CARBOPLUS, safety, zootechnical additives

CONTENTS

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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of feed additive shall submit an application in accordance with Article 7. In addition, Article 13(3) of that Regulation lays down that if the holder of an authorisation proposes changing the terms of the authorisation by submitting an application to the Commission, accompanied by the relevant data supporting the request for the change, the Authority shall transmit its opinion on the proposal to the Commission and the Member States.

The European Commission received a request from Kaesler Nutrition GmbH[2] for the authorisation of a new use and a modification of the current authorisation of the additive consisting of a preparation of xylanase (produced with *Komagataella phaffii* DSM 25376[3]) and β-glucanase (produced with *K. phaffii* DSM 26469[4]) (ENZY CARBOPLUS®) when used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, laying hens, minor avian species (game birds, ducks, geese, pigeons, sporting and ornamental birds), including laying birds, weaned piglets and minor weaned porcine species (category: zootechnical additive; functional group: digestibility enhancers). During the assessment, the applicant requested a change in the target species subject of application by restricting them to all poultry.[5]

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 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 13(3) (modification of the authorisation of a 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 17 February 2022.

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 feed additive consisting of preparation of xylanase (produced with *K. phaffii* DSM 25376) and β-glucanase (produced with *K. phaffii* DSM 26469) (ENZY CARBOPLUS®), when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

The additive is a preparation containing xylanase, produced with *K. phaffii* DSM 25376 and β-glucanase, produced with *K. phaffii* DSM 26469. EFSA issued one opinion on the safety and efficacy of this product when used in feed for avian species, weaned piglets and minor weaned porcine species (EFSA FEEDAP Panel, 2017a).

The additive is currently authorised for use in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, all avian species reared for laying or for breeding purposes other than chickens reared for laying, weaned piglets and minor porcine species (weaned) (4a28).[6]

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[7] in support of the authorisation request for the use of the feed additive consisting of a preparation of xylanase (produced with *K. phaffii* DSM 25376) and β-glucanase (produced with *K. phaffii* DSM 26469) (ENZY CARBOPLUS®) as a feed additive.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 17 February 2022 to 17 May 2022; the comments received were considered for the assessment.

[1]Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2]Kaesler Nutrition, Zeppelinstraße 3, 27472 Cuxhaven, Germany.
[3]Previously identified as *Komagataella pastoris* DSM 25376.
[4]Previously identified as *Komagataella pastoris* DSM 26469.
[5]Technical dossier/SIn_December 2023/Annex_I_FAD-2021-0064_SIn_231215 and 2024-01-11-Clarification provided.
[6]COMMISSION IMPLEMENTING REGULATION (EU) 2018/1090 of 31 July 2018 concerning the authorisation of a preparation of endo-1,4-beta-xylanase and endo-1,3(4)-betaglucanase produced by Komagataella pastoris (CBS 25376) and Komagataella pastoris (CBS 26469) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, all avian species reared for laying or for breeding purposes, weaned piglets and minor porcine species (weaned) (holder of the authorisation Kaesler Nutrition GmbH). OJ L 195, 1.8.2018, p. 23.
[7]FEED dossier reference: FAD-2021-0064.

The dossier was received on 19/4/2021, and the general information and supporting documentation are available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2021-00314.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substances in animal feed are valid and applicable for the current application.[8]

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the feed additive consisting of preparation of xylanase (produced with *K. phaffii* DSM 25376) and β-glucanase (produced with *K. phaffii* DSM 26469) (ENZY CARBOPLUS®) is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b), EFSA Statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (2021), Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2024).

3 | ASSESSMENT

The feed additive containing xylanase (IUBMB EC 3.2.1.8.) produced with *K. phaffii* DSM 25376 and β-glucanase (IUBMB EC 3.2.1.6) produced with *K. phaffii* DSM 26469 is currently authorised for use in chickens for fattening, chickens reared for laying, turkeys for fattening, all avian species reared for laying or for breeding and weaned piglets and minor weaned porcine species, as a zootechnical additive (functional group: digestibility enhancers). The applicant requested the modification of the terms of its authorisation for chickens for fattening and chickens reared for laying (reduction of the minimum recommended use level from 4250 xylanase enzyme activity (LXU) and 375 glucanase enzyme activity (LGU)/kg complete feed to 1400 LXU and 120 LGU/kg complete feed) and the request for an extension of use to all poultry at a minimum recommended level of 1400 LXU/kg and 120 LGU/kg feed. The solid and liquid forms of the additive will be hereafter referred to with their trade names ENZY CARBOPLUS® and ENZY CARBOPLUS® L, respectively.

3.1 | Characterisation

3.1.1 | Characterisation of the production organisms

The active substances in the additive are produced with two genetically modified production strains, *K. phaffii* DSM 25376, producing xylanase, and *K. phaffii* DSM 26469, producing glucanase. The strains are deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen.[10]

The production strains and their genetic modification were characterised in a previous opinion (EFSA FEEDAP Panel, 2017a). The strains have not been genetically modified further, but new information following the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (2018) and the EFSA Statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (2021) were submitted and is described below.

The production strains were previously allocated to the species *Komagataella pastoris*. With the data submitted in the current application, the production strains were identified as *K. phaffii* by alignment of the whole genome sequence (WGS) data to the well-known reference strain *K. phaffii* GS115. This was further confirmed by a phylogenetic analysis based on the *TEF1* (eukaryotic translation elongation factor 1 alpha) and *RPB1* (DNA-directed RNA polymerase) genes. The two production strains clustered with the well-known *K. phaffii* GS115 strain.[11]

[8]The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/document/download/7bce1ef2-502f-4304-8fe4-ce800add43ab_en?filename=FinRep-FAD-2013-0013-EnzyCarboplus.doc_.pdf.

[9]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.

[10]Technical dossier/Supplementary information December 2023/Annex IV.

[11]Technical dossier/Section II/Supplementary information September 2024/Reply to Addendum/Annex III.

Description of the genetic modifications

The scope of the genetic modifications is to increase the ability to produce xylanase and glucanase. The recipient strain is *K. phaffii* GS115.[12]

[redacted][13] All the genetic modifications were reported, including the intended and unintended ones, and no safety concerns were identified.

3.1.2 | Characterisation of the additive

The additive is authorised in two different formulations: solid (ENZY CARBOPLUS®) and liquid (ENZY CARBOPLUS® L). Both forms are authorised at a minimum guaranteed enzyme activity of 25,000 xylanase units (LXU)[14] and 2200 glucanase units (LGU)[15]/g of product.

ENZY CARBOPLUS® contains the two enzymes solid concentrates (1% xylanase and 0.5% glucanase) and 88.5% starch, with 10% moisture.[16]

ENZY CARBOPLUS® L contains the two enzymes liquid concentrates (1% xylanase and 0.5% glucanase), 15% glycerol, 5% sodium chloride, 0.30% sodium benzoate and 0.15% potassium sorbate, with 78.1% water.[17]

The two formulations of the additive under assessment have the same composition and method of manufacture as those considered in a previous application (EFSA FEEDAP Panel, 2017a). Therefore, all data on composition, purity and physico-chemical properties described earlier are also considered valid for this application. The applicant has provided new data on batch-to-batch variation, purity and physico-chemical properties, described below.

Analytical data to confirm the specifications set in the authorisation were provided for six batches of each formulation.[18] The average values of xylanase and glucanase for ENZY CARBOPLUS® were 37,211 (34,500–39,448) LXU/g and 3029 (2858–3150) LGU/g, respectively and for ENZY CARBOPLUS® L, 36,571 (33,900–39,950) LXU/g and 3514 (3168–3820) LGU/g, respectively.

Three batches of each formulation of the additive were analysed for impurities. The analysis included arsenic, cadmium, lead and mercury, which were all below their respective limits of quantification (LOQ) in both additive forms.[19] The analysis of aflatoxins (B1, G1, B2, G2) and T-2 toxin showed values below the LOQ in both forms. The analysis of zearalenone showed in the solid form values ranging from 24 to 26 μg/kg and in the liquid form values below the LOQ in two batches and 5.9 μg/kg in the third batch. The analysis of ochratoxin A showed values below LOQ in all batches tested, except for one batch of the liquid form, which showed a value of 0.78 μg/kg.[20]

Dioxins (polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F)), dioxin-like polychlorinated biphenyls (DL-PCBs) and non-DL PCBs were below the corresponding LOQs in all batches of the solid and liquid forms tested. The upper bound calculated as World Health Organization Toxic Equivalent (TEQ; van den Berg et al., 2006) was 0.17 ng WHO_{2005}-PCCD/F-TEQ/kg for dioxins, 0.3 ng WHO_{2005}-PCCD/F + PCB-TEQ kg for the sum of dioxins and DL-PCBs and 1.7 μg/kg for non-DL PCBs.

[12]Technical dossier/Section II/Supplementary information September 2024/Reply to Addendum/Annex IV, Annex V and Annex VI.

[13]Technical dossier/Section II/Supplementary information September 2024/Reply to Addendum/Annex_VII.

[14]One xylanase unit (LXU) is the amount of enzyme that releases one μmole of reducing sugar equivalents per minute (as xylose from birch xylan) at pH 5.5 and 50°C.

[15]One β-glucanase unit (LGU) is the amount of enzyme which liberates one μmole of reducing sugar equivalents per minute (as glucose from barley glucan) at pH 5.5 and 50°C.

[16]Technical dossier/Supplementary information December 2023/Annex_I_FAD-2021-0064_SIn_231215.

[17]Technical dossier/ Supplementary information December 2023/Annex_I_FAD-2021-0064_SIn_231215.

[18]Technical dossier/Supplementary information December 2023/Annex_II.

[19]Technical dossier/Section II/ Annex_II_1_4_1_3_PART_2 and Annex_II_1_4_1_4_PART_2; LOQ for Arsenic: 0.50 mg/kg, Cadmium: 0.20 mg/kg, Lead: 0.50 mg/kg, Mercury: 0.02 mg/kg.

[20]Technical dossier/Section II/Annex_II_1_4_1_3_PART_2 and Annex_II_1_4_1_4_PART_2; LOQ aflatoxins (B1, G1, B2, G2): 1 μg/kg; zearalenone: 5 μg/kg; ochratoxine A: 0.5 μg/kg; T-2 toxin: 5 μg/kg.

The microbiological contamination in three batches of each formulation was evaluated by analysing the counts of coliforms, Escherichia coli, yeasts, filamentous fungi and *Enterobacteriaceae*.[21] All values were < 10 colony-forming units (CFU)/g. *Salmonella* spp. was not detected in 25 g in three batches of either formulation.

The detected amounts of the impurities and microbial contamination described above do not raise safety concerns.

The absence of viable cells of the two production strains was determined in three batches of the solid and three batches of the liquid forms of the additive, each tested in triplicate.[22] For the liquid samples, 1 mL was plated on five non-selective agar plates and incubated for 5 days at 25°C. From the solid samples, only 0.5 g was tested. A positive control was included in the analysis. No colonies were detected on the plates. The amount tested for the solid formulation is not compliant with the Guidance (EFSA FEEDAP Panel, 2018). However, the manufacturing process of the solid and liquid formulations includes the same steps for its downstream processing up to the drying step followed to obtain the solid product.[23] Therefore, the Panel considers that the analysis results for the presence of viable cells in the liquid formulation would also apply to the solid formulation.

The absence of DNA from the two production strains was confirmed in three batches of the liquid form and three of the solid one, each tested in triplicate.[24] A PCR specific for each production strain was used, amplifying a 733 bp fragment specific for the glucanase production strain and an 808 bp fragment for the xylanase production strain. The analysis started with 1 g/mL of product. Considering the cell lysis efficiency and the sensitivity of the PCR method, the analysis was concluded to be adequate to reach the requested detection limit of 10 ng/g or 10 ng/mL of product. No DNA of the production strains was detected.

The applicant provided new data on the physico-chemical properties of the liquid formulation: pH was measured in six batches and ranged between 5.18 and 5.30; specific weight, surface tension and viscosity were measured in three batches and ranged from 10.73–10.74 kN/m^3 (at 25°C), 47.3–47.5 mN/m and 2.16–2.19 mPa·s (at 25°C), respectively.[25]

3.1.3 | Conditions of use

The additive is currently authorised as a zootechnical additive for use in feed for chickens for fattening and reared for laying at a minimum level of 4250 LXU/kg feed and 375 LGU/kg complete feed, and in turkeys for fattening and all avian species reared for laying or for breeding purposes other than chickens reared for laying at 1400 LXU/kg and 120 LGU/kg complete feed.

The applicant has asked to reduce the minimum recommended levels for chickens for fattening and chickens reared for laying to 1400 LXU/kg and 120 LGU/kg complete feed.

In addition, the applicant proposed the extension of use to all poultry at a minimum recommended level of 1400 LXU/kg and 120 LGU/kg feed.

3.2 | Safety

3.2.1 | Safety of the production microorganisms

The genetically modified strains DSM 25376 and DSM 26469 were previously allocated to the species *K. pastoris* (EFSA FEEDAP Panel, 2017a). In the context of the present application, the production strains were identified by WGS as belonging to the species *K. phaffii*, which also qualifies for the qualified presumption of safety (QPS) approach to safety assessment when used for production purposes (EFSA BIOHAZ Panel, 2023). The applicant provided information showing that the genetic modifications do not raise safety concerns. No viable cells or DNA of the production strains were detected in the final products. Therefore, the FEEDAP Panel concludes that the additive poses no safety concern regarding the genetically modified *K. phaffii* DSM 25376 and DSM 26469 strains.

3.2.2 | Safety for the target species, consumers and the environment

The safety aspects regarding the use of this additive in feed, including the safety for the consumers, the user and the environment, have been previously assessed (EFSA FEEDAP Panel, 2017a). The Panel concluded that the use of the product as a feed additive raises no concerns for consumers or the environment. Based on the tolerance trials submitted in chickens for fattening, turkeys for fattening and laying hens, the Panel concluded that the additive was safe for all avian species at 1400 LXU and 120 LGU/kg. Regarding the safety for the user, the Panel concluded that the additive in either formulation is not a skin or eye irritant or a dermal sensitiser but is considered a respiratory sensitiser.

[21]Technical dossier/Supplementary information December 2023/Annex_VI and SIn September_2024/ Annex_III.

[22]Technical dossier/Section II/Supplementary information September 2024/Reply to Addendum/Annex II, Annex VIII and Annex IX.

[23]Technical dossier/Section II/Supplementary information December 2024/Annex II 3 2 1 and Annex II 3 2 2.

[24]Technical dossier/Section II/Supplementary information September 2024/Reply to Addendum/Annex II, Annex VIII and Annex X.

[25]Technical dossier/Supplementary information December 2023/Annex_II and Annex_IX.

The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously, and considers that the modification of authorisation of use for chickens for fattening and reared for laying and the extension of use to all poultry for which the application is made would not have an impact on the safety aspects already evaluated.

3.3 | Efficacy

3.3.1 | Efficacy for chickens for fattening

Based on the data submitted in the context of a previous application, the Panel concluded that the additive had the potential to be efficacious in chickens for fattening and chickens reared for laying from 4250 LXU to 1400 LXU/kg complete feed (EFSA FEEDAP Panel, 2017a). In the current application, three long-term trials were submitted to support the efficacy at the newly proposed minimum use level (1400/120 LXU/LGU per kg feed) in chickens for fattening. Two of the trials[26] were previously assessed by the Panel and were considered to support the potential of the additive to improve the zootechnical performance of chickens for fattening when included in the feed at 1400/120 LXU/LGU per kg feed (EFSA FEEDAP Panel, 2017a).

The third study[27] submitted is a long-term trial aiming at evaluating the effect of the additive on the zootechnical performance, which includes a balance trial to assess the effects on energy utilisation. Thirty-six one-day-old Ross 308 chicks were individually housed in cages and randomly allocated to three experimental groups. The basal diets (starter, from day 1 to 21; grower, from day 22 to 35) based on maize, wheat, rye and soybean meal were either not supplemented (control) or supplemented with ENZY CARBOPLUS® (solid) to provide 700/60 or 1400/120 LXU/LGU per kg complete feed. The recovery rate of the additive in the feed was confirmed based on the analysis of the xylanase activity.[28] The experimental diets were offered ad libitum as pellets for 35 days. The mortality and health status of the animals were checked daily. The birds were weighed at the start of the trial. Thereafter, the zootechnical performance was monitored weekly. During five days of the starter (from day 14 to 18) and grower (from day 28 to 32) phases, excreta were collected (total collection method) and pooled per cage. Feed and excreta samples were analysed for dry matter and energy content. The apparent metabolisable energy (AME) of the diets was calculated. The experimental data were analysed with an analysis of variance, including diet as a fixed effect. Group means were compared with a Tukey test when a difference was observed. The cage was used as the experimental unit. The significance level was set at 0.05.

The Panel notes that the animals were individually caged during the whole duration of the trial, which is not aligned with current farming conditions in the European Union. Therefore, the zootechnical performance data were not considered to assess efficacy.

No bird died during the experiment. The chickens that received the additive at 1400/120 LXU/LGU per kg complete feed showed higher dietary AME than the control in the two periods evaluated (14.0 vs. 13.3 MJ/kg DM for starter; 15.2 vs. 14.2 MJ/kg DM for grower). Similarly, higher AME was observed in the birds receiving the additive in the grower phase at 700/60 LXU/LGU per kg complete feed (15.0 MJ/kg DM) compared to the control.

3.3.2 | Efficacy for laying hens

In the previous opinion, the Panel could not conclude on the efficacy of the additive in laying hens due to the lack of sufficient data (EFSA FEEDAP Panel, 2017a). In the current application, three short-term balance trials were submitted to support the efficacy on laying hens at the minimum use level (1400/120 LXU/LGU per kg complete feed). One of the trials[29] was already assessed by the Panel and showed higher metabolisable energy content of the diets in the supplemented group compared to the control group at the proposed level (EFSA FEEDAP Panel, 2017a).

The other two studies submitted (trials 2[30] and 3[31]) followed a similar experimental design. The details of the study design are provided in Table 1, and the main results are in Table 2.

[26]Technical dossier/Section IV/Annex IV 3.1 and 3.2.

[27]Technical dossier/Section IV/Annex IV 3.3.

[28]Starter/Finisher (LXU/kg feed): < 100/105 for control; 536/675 for the 700 LXU group; 821/1230 for the 1400 LXU group.

[29]Technical dossier/Section IV/Annex IV 2.3.

[30]Technical dossier/Section IV/Annex IV 2.1.

[31]Technical dossier/Section IV/Annex IV 2.2.

TABLE 1 Trial design and use level of the efficacy trials performed in laying hens.

Trial	N° of animals (animals × replicate) replicates × group	Breed (age)	Duration (adaptation/ collection)	Composition feed (form)	Groups (LXU/LGU per kg feed)	
					Intended	Analysed[(1)]
2	72 (1) 18	Lohmann Brown (23 weeks)	9 days/5 days	Wheat, rye, soybean meal, barley (mash)	0/0 700/60 1400/120 2100/180	< 100 730 1550 2370
3	72 (1) 18	Lohmann Brown (32 weeks)	9 days/5 days	Wheat, rye, soybean meal, barley (mash)	0/0 700/60 1400/120 2100/180	124 642 1460 2210

Abbreviations: LGU, glucanase enzyme activity; LXU, xylanase enzyme activity.

[(1)]The recovery rate of the additive in feed was confirmed based on the analytical xylanase activity. The glucanase activity was not measured.

In both trials, hens were individually housed in cages and randomly allocated to four groups (18 replicates per group) based on the inclusion rate of the additive. The basal diets were either not supplemented (control) or supplemented with ENZY CARBOPLUS® (solid) to provide 700/60, 1400/120 or 2100/180 LXU/LGU per kg complete feed. The recovery rate of the additive in the feed was confirmed based on the analytical xylanase activity (Table 2). The feed contained an external marker and was offered ad libitum in mash form for 14 days. The mortality and general health were monitored daily throughout the study. Body weight was recorded on the first day of the trial and, together with the feed consumption, monitored weekly (on days 7 and 14). The laying performance and the egg quality were monitored during the trial. From days 10 to 14, the excreta from each hen were collected (by the total collection method) and pooled. Feed and excreta samples were analysed for the content of the external marker, nitrogen and gross energy. The nitrogen-corrected AMEn was calculated. The experimental data were analysed with an analysis of variance, including diet as a fixed effect. When differences were found, group means were compared with the Tukey test. The significance level was set at 0.05.

No hen died in any of the trials. In both trials, the hens that received the additive at 1400/120 LXU/LGU per kg complete feed showed higher dietary AME corrected for nitrogen (AMEn) than the control. No effects were observed on the body weight, feed intake, laying performance and egg quality.

TABLE 2 Effects of ENZY CARBOPLUS on the nitrogen-corrected apparent metabolisable energy (AMEn) of laying hens.

Trial	Groups (LXU/LGU per kg feed)	AMEn (MJ/kg)
2	0/0	10.5^{a}
	700/60	10.7^{ab}
	1400/120	11.0^{bc}
	2100/180	11.0^{c}
3	0/0	10.9^{a}
	700/60	11.1^{ab}
	1400/120	11.4^{b}
	2100/180	11.2^{ab}

Abbreviations: LGU, glucanase enzyme activity; LXU, xylanase enzyme activity.

[a,b]Mean values within a trial and a column with a different superscript are significantly different $p < 0.05$.

3.3.2.1 | *Conclusions on the efficacy for all poultry*

In the previous application, the inclusion of the additive at 1400/120 LXU/LGU per kg complete feed showed potential to improve the zootechnical performance of chickens for fattening in two trials and to increase the metabolisable energy of the diet in one trial in laying hens. In the current application, one trial in chickens for fattening and two in laying hens support the potential of the additive to improve the dietary metabolisable energy when included in the feed at 1400/120 LXU/LGU per kg complete feed.

Taking all data together, the FEEDAP Panel concludes that the additive has the potential to be efficacious when included at 1400/120 LXU/LGU per kg complete feed for chickens for fattening and laying hens. The conclusion can be extrapolated to all poultry.

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[32] and Good Manufacturing Practice.

4 | CONCLUSIONS

The production strains *K. phaffii* DSM 25376 and DSM 26469 do not raise safety concerns concerning the genetic modifications. No viable cells or DNA of the production strains were detected in the final product. Therefore, the FEEDAP Panel concludes that the additive does not pose any safety concern regarding the production strain.

The use of the additive at 1400 LXU and 120 LGU/kg complete feed is considered safe for all poultry.

The use of the feed additive in all poultry under the proposed conditions of use is considered safe for consumers or the environment.

The Panel concluded that the additive in either formulation is not a skin or eye irritant or a dermal sensitiser but is considered a respiratory sensitiser.

The additive is considered to be efficacious in feedingstuffs for all poultry at 1400/120 LXU/LGU per kg complete feed.

ABBREVIATIONS

AME	apparent metabolisable energy
AMEn	nitrogen-corrected apparent metabolisable energy
BLAST	basic local alignment search tool
BW	body weight
CFU	colony forming unit
DL-PCBs	dioxin-like polychlorinated biphenyls
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LGU	glucanase enzyme activity
LOQ	limit of quantification
LXU	xylanase enzyme activity
NCBI	National Center for Biotechnology Information
PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofurans
PCR	polymerase chain reaction
QPS	qualified presumption of safety
TEQ	World Health Organization Toxic Equivalent
WGS	whole genome sequencing
WHO	World Health Organization

REQUESTOR

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PANEL MEMBERS

Roberto Edoardo Villa, Giovanna Azimonti, Eleftherios Bonos, Henrik Christensen, Mojca Durjava, Birgit Dusemund, Ronette Gehring, Boet Glandorf, Maryline Kouba, Marta López-Alonso, Francesca Marcon, Carlo Nebbia, Alena Pechová, Miguel Prieto-Maradona, Ilen Röhe and Katerina Theodoridou.

LEGAL NOTICE

[32]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.

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