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SCIENTIFIC OPINION

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Safety of a feed additive consisting of *Duddingtonia flagrans* NCIMB 30336 (BioWorma®) for all grazing animals (International Animal Health Products Pty Ltd)

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Abstract

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 of a feed additive consisting of *Duddingtonia flagrans* NCIMB 30336 (BioWorma®) for all grazing animals. The safety and efficacy of the additive have been already assessed previously, however the FEEDAP Panel could not conclude on the safety of the additive for the target species and the consumers due to the limitations in the dataset provided. For the current assessment, the applicant submitted a new tolerance trial in dairy cows and new toxicological studies. After the assessment of the new data submitted, the FEEDAP Panel concluded that the use of the feed additive in animal nutrition under the conditions of use proposed is of no concern for dairy cows. This conclusion can be extrapolated to all dairy bovines, ovines and caprines, but not to fattening and rearing animals of those species. Due to the lack of data, the FEEDAP Panel cannot conclude on the safety of the additive for other grazing species/categories. The FEEDAP Panel concluded that the additive is safe for the consumers.

KEYWORDS

Duddingtonia flagrans, eelworm, grazing animals, nematodes, safety

CONTENTS

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The Applicant, International Animal Health Products Pty Ltd, represented in the EU by GAB Consulting GmBH, is seeking a Community authorisation of *Duddingtonia flagrans* NCIMB 30336[2] (BioWorma®) as a feed additive to be used as gut flora stabilisers and other zootechnical additives for all grazing animals (Table 1).

TABLE 1 Description of the substances.

Category of additive	Zootechnical additives
Functional group of additive	Gut flora stabilisers and other zootechnical additives
Description	*Duddingtonia flagrans* IAH 1297 (BioWorma®)
Target animal category	All grazing animals
Applicant	International Animal Health Products Pty Ltd, represented in the EU by GAB Consulting GmBH
Type of request	New opinion

On July 2020, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) of the European Food Safety Authority (EFSA), in its opinion on the safety and efficacy of the product, could not conclude on the safety of the additive.

The Commission gave the possibility to the applicant to submit supplementary information and data in order to complete the assessment and to allow a revision of the EFSA's opinion. The new data have been received on 21 March 2023.

In view of the above, the Commission asks EFSA to deliver a new opinion on *Duddingtonia flagrans* IAH 1297 (BioWorma®) as a feed additive for all grazing animals based on the supplementary information and data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2 | Additional information

The additive consisting of on *Duddingtonia flagrans* NCIMB 30336 (BioWorma®) is not authorised as a feed additive in the EU.

The FEEDAP Panel has adopted two opinions on the safety and efficacy of the additive under assessment (EFSA, 2006; EFSA FEEDAP Panel et al., 2020).

2 | DATA AND METHODOLOGIES

2.1 | Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[3] to a previous application on the same product.[4] The dossier was received on 9/8/2023 and the general information and supporting documentation are available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00742.

In accordance with Article 38 of the Regulation (EC) No 178/2002[5] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[6] a non-confidential version of the supplementary information has been published on Open.EFSA.

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' (elicitation) knowledge, to deliver the present output.

[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]The applicant identified the strain as: *Duddingtonia flagrans* IAH 1297, but in this opinion it will be referred to using its deposition number *Duddingtonia flagrans* NCIMB 30336.

[3]Dossier reference: EFSA-Q-2022-00742.

[4]Dossier reference: FAD-2016-0067.

[5]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[6]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety of the feed additive consisting of *D. flagrans* NCIMB 30336 (BioWorma®) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel et al., 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel et al., 2017b).

3 | ASSESSMENT

D. flagrans NCIMB 30336 (BioWorma®) is intended for use as a zootechnical feed additive (functional group: gut flora stabiliser and other zootechnical additives) to control pathogenic nematodes (eelworm) in the soil, with subsequent benefits for ruminants, horses and other grazing animals. *D. flagrans* (Dudd) Cooke belongs to a group of nematophagous fungi that physically entrap nematodes by means of a specialised adhesive hyphal net. The species is widely distributed and has been isolated from pasture soils in many countries worldwide.

The additive is specified to contain a minimum of 5×10^5 chlamydospores/g of additive (EFSA FEEDAP Panel et al., 2020). The additive is intended for use in all grazing animals (pigs (all categories), bovines (all categories), sheep (all categories), goats (all categories), rabbits (all categories), horses (all categories) and all other ruminant species: calves/kids of species in the family *Cervidae* (deer, etc.) and *Camelidae* (alpacas, etc)) at a daily dose of 1.5 g additive per 25 kg body weight (bw) (equivalent to 60 mg/kg bw per day) to be incorporated directly in feed or via a premixture. This approximates to 3×10^4 chlamydospores/kg bw per day. No withdrawal period is proposed.

The additive was fully characterised, and its efficacy evaluated in the previous assessment (EFSA FEEDAP Panel et al., 2020) which concluded that the additive reduces the number of parasitic nematodes on pasture to the benefit of grazing animals when used at the recommended application rate of 3×10^4 chlamydospores/kg bw per day. In the same opinion, the Panel concluded that the additive is safe for the environment and that it is not irritant to skin and eyes but is irritant to the respiratory tract and a respiratory sensitiser. No conclusion could be drawn on its skin sensitisation potential. However, the FEEDAP Panel could not conclude on the safety of the additive for the target species and for the consumers due to limitations identified in the dataset provided by the applicant in support of the safety of the additive (EFSA FEEDAP Panel et al., 2020). The applicant has provided new data to address the gaps identified in the previous opinion, which are assessed below.

3.1 | Safety

3.1.1 | Safety for the target species

In the previous evaluation, the FEEDAP Panel could not conclude on the safety of the additive for the target species due to the limitations in the experimental design in the three tolerance trials provided (e.g. no use level tested, lack of experimental replication, wrong experimental unit).

In the current dossier, the applicant provided a new tolerance trial conducted in lactating dairy cows[8] to support the safety for the target animals.

A total of 30 cows (Holstein/Friesian, in the first third of lactation and weighing 520–764 kg bw at start) were housed in open paddocks and grazed all together as a single herd. Cows were randomly allocated into three groups (representing 10 cows per group) balanced by body weight. A complementary feed[9] was provided individually, during milking, at a rate of 6 kg/day until day 21 and then 8 kg/day until the end of the study. Additionally, 1 g protein feed meal[10]/kg bw was individually provided to the animals, on top of the complementary feed, which was either not supplemented (control) or supplemented with BioWorma® at 3×10^4 spores/kg bw (1×) or 3×10^5 spores/kg bw (10×). The additive was offered for 56 days.

Health status of the animals was monitored at start and throughout the experiment. Individual body weight was measured before starting (day-3) and then on days 14, 28 and 56. Milk yield was recorded on days 0/1, 14/15, 28/29, 42/43 and 56/57. Milk composition (fat, protein, somatic cell count, urea, lactose) was assessed on days 0/1, 28/29, 42/43 and 56/57. Blood samples were taken from all animals before start (day-3) and on days 14, 28 and 56 and analysed for haematology and biochemical parameters.[11]

Data were compared using repeated-measures ANOVA including diet, time and the interaction diet×time as fixed effects. Group means were compared with Tukey's test. The significance level was set at 0.05.

[7]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.

[8]Annex_3.1.1_04_addsub_marked.pdf.

[9]Complementary feed composition: mineral mix, sugar, salt, oil, canola, magnesium phosphate, crude oils, calcium, tritacale.

[10]Commercial protein meal composed of oilseed meal, fish oil, molasses, vitamins and minerals.

[11]Blood parameters analysed: glucose, urea, creatinine, total protein, albumin, globulin, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), cholesterol, magnesium, calcium, phosphate, sodium, potassium, chloride, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin, concentration (MCHC), white cell differential count.

No health issue was recorded during the experiment. After 56 days of supplementation, no differences were observed between any treatment in body weight (−0.007 kg/day), milk yield (25.1 kg/day) and any of the blood haematology and biochemistry parameters analysed. Regarding milk quality, the only difference observed was a lower protein content in the 1× (2.95%) and 10× (2.91%) groups compared to the control (3.14%).

Conclusions on the safety for the target species

The additive is considered to be safe for dairy cows at the recommended dose (3×10^4 spores/kg bw) with a margin of safety of 10. This conclusion can be extrapolated to all dairy bovines, ovines and caprines, but not to fattening and rearing animals of those species. Due to the lack of data, the FEEDAP Panel cannot conclude on the safety of the additive for other grazing species/categories.

3.1.2 | Safety for the consumer

In the previous evaluation (EFSA FEEDAP Panel et al., 2020), the FEEDAP Panel could not conclude on the safety of the additive for the consumer due to the limited dataset provided comprising an acute toxicity study in rats and an *in-silico* assessment of the potential toxicity of a secondary metabolite of the fungus (flagranone A).

In the current submission, the applicant has provided genotoxicity studies and a 90-day study conducted with the additive under assessment, which are assessed below.

3.1.2.1 | *Toxicological studies*

Bacterial reverse mutation test

In order to evaluate the potential of *D. flagrans* NCIMB 30336 to induce gene mutations in bacteria, an Ames test was performed in *Salmonella* Typhimurium strains TA1537, TA1535, TA98, TA100 and *Escherichia coli* strain WP2 uvrA according to the OECD TG 471 (2022).[12] The study was claimed to be Good Laboratory Practice (GLP) compliant. The test item resulted insoluble in aqueous and organic solvents compatible with the test system. Thus, extracts in pure ethanol (> 99%) were obtained by incubation of the test item for 72 h at 37°C, followed by centrifugation to separate the test item from the ethanol extract. The supernatant was used for application to the test system. The extraction procedure was in accordance with ISO 10993-12.

Six concentrations ranging from 10% to 100% of the extracts/plate were tested in the presence and absence of metabolic activation (S9 mix). No toxicity was induced up to the highest concentration tested. No increase in the number of revertant colonies was observed in any tester strain and experimental condition.

In vitro *mammalian cell micronucleus test*

The test item *D. flagrans* NCIMB 30336, extracted in 1.0% DMSO, was assessed for its potential to induce micronuclei in human lymphocytes in vitro in two independent experiments, according to OECD 487.[13] The study was claimed GLP compliant. The test item extract preparation was done in DMSO, with final concentrations of 0.6%, 0.8% and 1.0% of the test item extract.

In the initial cytotoxicity testing in the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration (1.0% test item extract), which showed precipitation in the absence of S9 mix after 3 h treatment.

Short term exposure (3 h) in the presence and in the absence of metabolic activation and continuous exposure (28 h) in the absence of metabolic activation and duplicate cultures were used for the study. Cytochalasin B was added after 3 h exposure in the short treatment cultures and together with the treatment in long term culture; cells were harvested at 28 h after exposure. For each treatment condition, 2000 binucleated cells were scored. The study design included concurrent positive and negative (solvent or vehicle) controls, both with and without metabolic activation. In the absence and presence of S9 mix, no significant increases in the frequencies of micronucleated cells were observed after treatment with the test item extract.

The Panel concludes that, under the study conditions, there were no clastogenic and aneugenic effects of the test item.

90-day repeated dose oral toxicity study

Groups of 10 Wistar rats of each sex were given *D. flagrans* NCIMB 30336 diluted in Phosphate Buffered Saline and Tween 80 in ratio of 99.95:0.05 by gavage daily for 90 days at doses of 0, 100, 300 or 1000 mg/kg bw per day (corresponding to 6×10^5, 1.8×10^6 and 6×10^6 spores/kg bw per day, respectively). The study was conducted according to OECD TG 408 and claimed to be GLP compliant.[14]

[12] Annex_3.2.2.2.1_02_addsub_marked.pdf.
[13] Annex_3.2.2.2.2_01_addsub_marked.pdf.
[14] Annex_3.2.2.3_02_addsub_marked.pdf.

No adverse effects were reported, apart from some inflammatory lesions in the lungs of all treated groups, which are considered to have been associated with mechanically induced reflux of the test item solutions after gavage.

The FEEDAP Panel considered that the no observed adverse effect level (NOAEL) was at the highest dose tested of 1000 mg/kg bw per day (corresponding to 6×10^6 spores/kg bw per day).

3.1.2.2 | *Conclusions on safety for the consumer*

The FEEDAP Panel considers that, based on the toxicological studies provided, the use of the additive in animal nutrition under the proposed conditions of use is safe for the consumers.

4 | CONCLUSIONS

The additive is considered to be safe for dairy cows at the recommended dose (3×10^4 spores/kg bw). This conclusion can be extrapolated to all dairy bovines, ovines and caprines, but not to fattening and rearing animals of those species. Due to the lack of data, the FEEDAP Panel cannot conclude on the safety of the additive for other grazing species/categories.

The FEEDAP Panel concludes that the use of the feed additive in animal nutrition, under the conditions of use proposed, is of no concern for the consumer safety.

ABBREVIATIONS

bw	body weight
DM	dry matter
FCR	feed conversion ratio
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
MW	molecular weight
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

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