



ADOPTED: 22 January 2019

doi: 10.2903/j.efsa.2019.5604

Efficacy of a preparation of algae interspaced bentonite as a feed additive for all animal species

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) was asked to deliver a scientific opinion on the efficacy of a preparation of algae interspaced bentonite when used as aflatoxin B1 (AfB1) binder for all animal species; the additive is composed of bentonite feed grade and algae belonging to *Ulva* spp. The European Commission request follows an opinion of the FEEDAP Panel published in 2016; in that opinion, the FEEDAP Panel could not conclude on the efficacy of the additive. The applicant has submitted additional information to allow the FEEDAP Panel to complete its assessment; these additional data, related to the efficacy of the additive, are the subject of this opinion. In the efficacy study provided, the use of the additive reduced the AfM1 in milk, although this effect seemed to be not consistent over time; this study presented some weaknesses and limitations (incomplete data analysis, reporting and lack of determination of the active substance of the additive in feed) which do not allow the FEEDAP Panel to draw any conclusion on the efficacy of the additive. Due to insufficient evidence, the FEEDAP Panel cannot conclude on the efficacy of the algae interspaced bentonite as a technological feed additive for all animal species.

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Keywords: technological additives, mycotoxin binder, bentonite, algae, *Ulva* spp., efficacy

Requestor: European Commission

Question number: EFSA-Q-2018-00429

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Acknowledgements: The EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) wishes to thank the following for the support provided to this scientific output (in alphabetical order of the last name): Montserrat Anguita, Jaume Galobart, Lucilla Gregoretti, Orsolya Holczknecht and Niovi Kordali.

Suggested citation: EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Kouba M, Kos Durjava M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechová A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Chesson A, Gropp J, Martelli G, Renshaw D, López-Gálvez G and Mantovani A, 2019. Efficacy of a preparation of algae interspaced bentonite as a feed additive for all animal species. EFSA Journal 2019;17(2):5604, 7 pp. <https://doi.org/10.2903/j.efsa.2019.5604>

ISSN: 1831-4732

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Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the European Commission	4
1.2. Additional information.....	4
2. Data and methodologies.....	4
2.1. Data.....	4
2.2. Methodologies.....	4
3. Assessment.....	5
3.1. Efficacy	5
4. Conclusions.....	6
Documentation provided to EFSA	6
Chronology	6
References.....	6
Abbreviations	7

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

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

The applicant, OLMIX SA, is seeking a Community authorisation of preparation of algae interspaced bentonite as a feed additive to be used as a substance for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action for all animal species (Table 1).

Table 1: Description of the substances

Category of additive	Technological additive
Functional group of additive	Substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action
Description	Preparation of algae interspaced bentonite
Target animal category	All animal species
Applicant	OLMIX SA
Type of request	New opinion

On 21 November 2016, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the preparation of algae interspaced bentonite as a feed additive for all animal species, could not conclude on the efficacy of the product due to the lack of *in vivo* studies.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data have been received on 12 February 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion on preparation of algae interspaced bentonite as a feed additive for all animal species based on the additional data submitted by the applicant.

1.2. Additional information

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) adopted in 2016 an opinion on the safety and efficacy of the preparation of algae interspaced bentonite as a technological feed additive for all animal species (EFSA FEEDAP Panel, 2016). In that opinion, the Panel could not conclude on the efficacy of the additive since no adequate *in vivo* studies were available.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information¹ to a previous application on the same product.²

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of algae interspaced bentonite is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on technological additives (EFSA FEEDAP Panel, 2012) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a,b,c).

¹ FEED dossier reference: FAD-2018-0005.

² FEED dossier reference: FAD-2014-0047.

3. Assessment

The additive under assessment is composed of bentonite and algae belonging to *Ulva* spp. It is proposed to be used as a technological additive (functional group: substances for reduction of the contamination of feed by mycotoxins) in all animals with the aim of reducing the contamination of feed by mycotoxins.

The preparation of algae interspaced bentonite is intended to be used in premixtures and feedingstuffs for all animal species and categories at a recommended dose of 10–125 mg/kg complete feed. No withdrawal period is foreseen.

In a previous opinion of the FEEDAP Panel on the same additive (EFSA FEEDAP Panel, 2016), the Panel could not conclude on the efficacy of the additive since no adequate *in vivo* studies were available.

The applicant has submitted additional information related to the efficacy of the additive and this new information is the subject of this opinion.

3.1. Efficacy

The applicant provided a short-term efficacy study on dairy cows.³ A total of 24 Holstein lactating cows (half primiparous and half multiparous; on average 167 days in milk; 600 kg body weight (BW) and 29.6 kg milk yield/day) were used and housed indoors on free-stalls. The cows were allocated, according to a complete randomised block design, to the following three experimental treatments, each comprising eight animals:

- T1 (control): in which the basal diet was artificially contaminated with aflatoxin (Af) B1 (AfB1) at a level of 5.6 µg/kg dry matter (DM) (through the concentrate),
- T2: in which the control diet was supplemented with the additive at 10 mg/kg complete feed; the additive was diluted in calcium carbonate used as a carrier (concentration of the additive not confirmed by analysis), and
- T3: in which the control diet was supplemented only with the carrier.

Cows were identified with an electronic ear tag and fed twice daily. Animals received a total mixed ration (TMR; 7.0 MJ net energy of lactation/kg, and 148 g crude protein (CP), 322 g neutral detergent fibre and 469 g non-fibre carbohydrates/kg DM) mainly based on maize (20%), alfalfa hay (12%) and soya bean meal (10%). Experimental feeds were prepared every three days. The study lasted 21 days.

AfB1, AfB2, AfG1 and AfG2 were determined in the basal uncontaminated feed at the start of the trial (all values being < 1 µg/kg). After the AfB1 addition to the diet, three series of analysis were carried out on one frozen sample per treatment of the artificially contaminated TMR to assess the concentration of AfB1 in feed. The results indicated that AfB1 levels in the artificially contaminated diets (4.0–4.3 µg AfB1/kg TMR at 12% moisture) did not exceed the limit set up by legislation. No analyses were performed to confirm the presence of the additive in the TMR.

AfM1 in milk and AfB1 in blood were monitored for one week starting on day 15 of trial (two weeks after the beginning of the experiment). Milk AfM1 was analysed on individual composite (morning and evening milkings) milk samples on days 15, 16, 17, 18, 19, 20 and 21 for groups T1 and T2 and on days 15, 17, 19 and 21 for T3 cows.⁴ Blood AfB1 was analysed in samples taken from all cows on days 15, 17 and 19 of the experiment.

For milk yield, milk composition (fat and protein; analysed on one bulk sample per cow per day), body weight, dry matter intake (DMI) and feed efficiency (FE), the observations started 6 days before the experimental diets were provided.

An analysis of variance (ANOVA) was done with the data using a mixed model for repeated measurements considering the treatment, parity of the cow (grouped in two classes primiparous and multiparous) and day of measurement as fixed effects and their interactions. Day was used as the repeated measure and the autoregressive structure of covariance was used. Data on the aflatoxin content in milk were root transformed due to non-normal distribution.

All animals were in good health with the exception of one cow from treatment T2 which was removed from the study on day 10 due to an accident; an outlier cow in the same group was excluded.

³ Technical Dossier/Annex2C_Final_Report_AFB1_Dairy_Cows.pdf.

⁴ The discrepancy in the number of samples taken from the different groups, leading to a lower number of samples for T3 group was due to the fact that this latter group (T3), containing the carrier alone, was included only for informative purposes (aiming to demonstrate the absence of effects due to the carrier). Technical Dossier/Supplementary information.

The performance parameters tested of cows – recorded over a 27-day period – were unaffected by treatments; the values found for the control group were the following: dry matter intake 23.0 kg DM/day; milk yield 28.7 kg/day; milk fat 3.88%; BW 594 kg. The report of the statistical analysis of the data from the 21-day treatment period was not provided.

Even if performance data were statistically analysed for the 27-day period (21 days of treatments plus 6 days before the start of the treatments, see above), no statistical analysis of milk production data relevant to the comparison between the groups during the last experimental week (days 15–21) – in which blood and milk samples were collected to assess AfB1 and AfM1 concentrations, respectively – was provided.⁵

No differences were observed between AfM1 content of milk produced on days 15, 17, 19 and 21 of trial by control cows and animals receiving the carrier alone (29.4 vs 34.3 ng/L for T1 and T3, respectively). The comparison carried out on milk samples daily collected for groups T1 and T2 indicated a significant reduction of AfM1 in milk deriving from cows receiving the complex containing the carrier plus the additive (31.6 vs 25.7 ng/L for T1 and T2, respectively; p < 0.05). A significant interaction was identified when analysing the data as treatment × day, thus showing a different behaviour of the effect of the treatment along time.

AfB1 concentration was not detected in any of the serum samples that were analysed (< 0.2 µg/L).

4. Conclusions

In the efficacy study provided, the use of the additive reduced the AfM1 in milk, although this effect seemed to be not consistent over time. This study presented some weaknesses and limitations (incomplete data analysis, reporting and lack of determination of the active substance of the additive in feed) which do not allow the FEEDAP Panel to draw any conclusion on the efficacy of the additive.

Due to insufficient evidence, the FEEDAP Panel cannot conclude on the efficacy of the algae interspaced bentonite as a technological feed additive for all animal species.

Documentation provided to EFSA

- 1) Safety and efficacy of a preparation of algae interspaced bentonite as a feed additive for all animal species. January 2018. Submitted by Olmix SA.
- 2) Safety and efficacy of a preparation of algae interspaced bentonite as a feed additive for all animal species. Supplementary information. August Submitted by Olmix SA.

Chronology

Date	Event
13/2/2018	Dossier received by EFSA
22/5/2018	Reception mandate from the European Commission
2/8/2018	Request of supplementary information to the applicant – Scientific assessment suspended. <i>Issues: efficacy</i>
31/8/2018	Reception of supplementary information from the applicant – Scientific assessment re-started
22/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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⁵ Technical Dossier/Supplementary information.

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Abbreviations

af	aflatoxin
ANOVA	analysis of variance
BW	body weight
CP	crude protein
DM	dry matter
DMI	dry matter intake
FE	feed efficiency
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
TMR	total mixed ration