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Efficacy of a feed additive consisting of *Bacillus subtilis* FERM BP-07462, *Enterococcus lactis* FERM BP-10867 and *Clostridium butyricum* FERM BP-10866 (BIO-THREE®) for chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding (TOA BIOPHARMA Co., Ltd.)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Durjava, Birgit Dusemund, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Roberto Edoardo Villa, Ruud Woutersen, Noël Dierick, Montserrat Anguita, Matteo L Innocenti, Jordi Ortuño, Jordi Tarres-Call and Joana P Firmino

Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the efficacy of *Bacillus subtilis* FERM BP-07462, *Enterococcus lactis* FERM BP-10867 and *Clostridium butyricum* FERM BP-10866 (BIO-THREE®) as a zootechnical feed additive to be used as a gut flora stabiliser for chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding. In a previous opinion, the FEEDAP Panel could not conclude on the efficacy of BIO-THREE® for the target species at the proposed conditions of use. The applicant has provided a new study in chickens for fattening as supplementary information to support the efficacy of BIO-THREE® for the target species. Considering the previously submitted studies and the new submitted trial, the Panel concluded that the additive is efficacious for chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding under the proposed conditions of use.

Keywords: zootechnical additives, gut flora stabiliser, BIO-THREE®, efficacy, chickens, turkeys, all avian species

Requestor: European Commission

Question number: EFSA-Q-2023-00186

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Declaration 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.

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1. Introduction

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

Regulation (EC) No 1831/2003 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, TOA BIOPHARMA Co., Ltd., Japan, represented in EU by TOA BIOPHARMA Co., Ltd., Europe Representative Office, is seeking a Community authorisation of *Bacillus subtilis* TO-A (BS), *Enterococcus faecium* T-110 (EF), *Clostridium butyricum* TO-A (CB) as a feed additive to be used as gut flora stabilisers for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species (Table 1).[1]

Table 1: Description of the additive

Category of additive	Zootechnical additives
Functional group of additive	Gut flora stabilisers
Description	*Bacillus subtilis* TO-A (BS), *Enterococcus faecium* T-110 (EF), *Clostridium butyricum* TO-A (CB)
Target animal category	Chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species
Applicant	TOA BIOPHARMA Co., Ltd., Japan, represented in EU by TOA BIOPHARMA Co., Ltd., Europe Representative Office
Type of request	New opinion

On 4 May 2022, 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 efficacy 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 7 March 2023.

In view of the above, the Commission asks EFSA to deliver a new opinion on *Bacillus subtilis* TO-A (BS), *Enterococcus faecium* T-110 (EF), *Clostridium butyricum* TO-A (CB) as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and minor poultry species based on the additional data submitted by the applicant, in accordance with Article 29(1)(a) of Regulation (EC) No 178/2002.

1.2. Additional information

The additive under assessment is a preparation containing viable spores/cells of *B. subtilis* FERM BP-07462, *E. lactis* (previously identified as *E. faecium*) FERM BP-10867 and *C. butyricum* FERM BP-10866 (BIO-THREE®). EFSA issued an opinion on the safety and efficacy of this product when used in feed for chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding (EFSA FEEDAP Panel, 2022).

The additive is currently not authorised as a feed additive in the European Union.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of supplementary information[2] to a previous application on the same product.[3] The dossier was received on 3/4/2023

[1] During the assessment it was clarified with the EC that the target species should be specified as follow: chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, all avian species for rearing/fattening to slaughter and all avian species reared for laying or breeding to point of lay.
[2] Dossier reference: EFSA-Q-2023-00186.
[3] Dossier reference: FAD-2020-0058.

and the general information and supporting documentation are available on Open.EFSA at https://open.efsa.europa.eu/questions/EFSA-Q-2023-00186.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] 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,[5] a non-confidential version of the supplementary information has been published on Open.EFSA.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Bacillus subtilis* FERM BP-07462, *Enterococcus lactis* FERM BP-10867 and *Clostridium butyricum* FERM BP-10866 (BIO-THREE®) is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The product under assessment is a preparation of viable spores/cells of *B. subtilis* FERM BP-07462, *E. lactis* FERM BP-10867 and *C. butyricum* FERM BP-10866 intended for use as a zootechnical additive (functional group: gut flora stabiliser) in feed and water for drinking for chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding. The minimum inclusion level is 2.0×10^7 *B. subtilis* FERM BP-07462 colony forming unit (CFU)/kg, 2.0×10^8 *E. lactis* FERM BP-10867 CFU/kg and 2.0×10^7 *C. butyricum* FERM BP-10866 CFU/kg complete feed, or of 1.0×10^7 *B. subtilis* FERM BP-07462 CFU/L, 1.0×10^8 *E. lactis* FERM BP-10867 CFU/L and 1.0×10^7 *C. butyricum* FERM BP-10866 CFU/L of water for drinking. It will hereafter be referred to as BIO-THREE®, its trade name.

In its previous opinion (EFSA FEEDAP Panel, 2022), the FEEDAP Panel concluded that the additive is considered safe for the target species, consumers and the environment. Regarding the safety for the user, BIO-THREE® was considered as non-irritant to the skin and eyes, but as a respiratory sensitiser. No conclusions were drawn on the potential of the additive to cause skin sensitisation. The FEEDAP Panel could not conclude on the efficacy of BIO-THREE® for the target species. Additionally, the FEEDAP Panel concluded that BIO-THREE® is compatible with diclazuril, decoquinate and halofuginone, but no conclusions could be drawn on the compatibility with monensin sodium, salinomycin sodium, narasin, robenidine hydrochloride and maduramicin ammonium.

3.1. Efficacy

In its previous assessment, the FEEDAP Panel could not conclude on the efficacy of BIO-THREE® for the target species, since the three independent studies considered for the assessment, showing positive effects of the supplementation with the additive on the performance of chickens for fattening, were performed in the same trial location within the EU, which does not comply with the requirements of Regulation (EC) No 429/2008 and the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

In the current submission the applicant has provided one trial in chickens for fattening, conducted in a different location than the previous, aimed at demonstrating the effect of the additive on the zootechnical performance.[7]

A total of 800 one-day-old male chickens for fattening (Ross 308) were distributed in a blocked design into 100 pens, in groups of eight animals and allocated to two groups (50 replicate pens per treatment). Three basal diets (starter from 1–14 day, grower from 15–28 day and finisher from 29–42 day), based on wheat, soyabean meal and maize, were either not supplemented (control) or

[4] 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, pp. 1–48.
[5] Decision is available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.
[6] 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.
[7] Annex_IV_3_9_efficacy_broilers.

supplemented with BIO-THREE® to provide 2.0×10^7 *B. subtilis* FERM BP-07462 CFU/kg, 2.0×10^8 *E. lactis* FERM BP-10867 CFU/kg and 2.0×10^7 *C. butyricum* FERM BP-10866 CFU/kg complete feed (confirmed by analysis).[8] The experimental diets were offered on *ad libitum* basis in mash form for 42 days.

Mortality and health status were checked every day and dead animals were necropsied. Body weight on days 1, 14, 28 and 42 and feed intake upon every diet change were recorded on a pen basis. The average daily feed intake, average daily gain and feed-to-gain ratio were calculated and corrected for mortality.

The zootechnical performance data were analysed with an analysis of variance via a General Linear Model with the diet as a fixed effect. The pen was considered the experimental unit. Significance level was set at 0.05.

Main results are reported in Table 2. Mortality and culling rates were in line with commercial standards and no differences were observed between groups. The chickens receiving the feed supplemented with BIO-THREE® at the minimum recommended level for 42 days showed an improved feed-to-gain ratio compared to the control group. No other difference was observed in any other performance parameter between groups.

Table 2: Effects of BIO-THREE® on the performance of chickens for fattening (1–42 days)

Groups *B. subtilis*/*E. lactis*/*C. butyricum* (CFU/kg feed)	Daily feed intake (g)	Final body weight (g)	Average daily weight gain (g)	Feed to gain ratio	Mortality and culling (%)
0/0/0	96.6	2,808	65.1	1.48[b]	3.5
$2.0 \times 10^7/2.0 \times 10^8/2.0 \times 10^7$	96.1	2,839	65.9	1.46[a]	3.0

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

Considering the overall results of the four studies submitted in the previous Opinion and in the current dossier, the FEEDAP Panel concludes that BIO-THREE® is efficacious in chickens for fattening at the minimum inclusion levels of 2.0×10^7 *B. subtilis* FERM BP-07462 CFU/kg, 2.0×10^8 *E. lactis* FERM BP-10867 CFU/kg and 2.0×10^7 *C. butyricum* FERM BP-10866 CFU/kg. The conclusions in feed can be applied for the use of the additive in water for drinking under the proposed conditions of use (1.0×10^7 *B. subtilis* FERM BP-07462 CFU/L, 1.0×10^8 *E. lactis* FERM BP- 10867 CFU/L and 1.0×10^7 *C. butyricum* FERM BP-10866 CFU/L). These conclusions are extended to chickens reared for laying and extrapolated to turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding.

4. Conclusions

Considering the previously submitted studies and the new submitted trial, the FEEDAP Panel concludes that the additive is efficacious for chickens for fattening and reared for laying, turkeys for fattening and reared for breeding, and all avian species for rearing/fattening or reared for laying/breeding under the proposed conditions of use.

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[8] Starter (CFU/kg feed): 2.6×10^7 *B. subtilis*, 1.1×10^7 *E. lactis*, 1.8×10^7 *C. butyricum*; Grower (CFU/kg feed): 3.0×10^7 *B. subtilis*, 1.2×10^7 *E. lactis*, 1.6×10^7 *C. butyricum*. The Panel notes that in both diets the content in *E. lactis* was 1-log lower than the intended concentration.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Azimonti, G, Bampidis, V, Bastos, ML, Christensen, H, Dusemund, B, Fašmon Durjava, M, Kouba, M, López-Alonso, M, López Puente, S, Marcon, F, Mayo, B, Pechová, A, Petkova, M, Ramos, F, Sanz, Y, Villa, RE, Woutersen, R, Brantom, P, Maradona, MP, Tosti, L, Anguita, M, Brozzi, R, Galobart, J, Pizzo, F, Revez, J, Ortuño, J, Tarrés-Call, J and Pettenati, E, 2022. Scientific Opinion on the safety and efficacy of a feed additive consisting of *Bacillus subtilis* FERM BP-07462, *Enterococcus lactis* FERM BP-10867 and *Clostridium butyricum* FERM BP-10866 (BIO-THREE®) for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding, all avian species for rearing/fattening to slaughter and all avian species reared for laying or breeding to point of lay (TOA BIOPHARMA Co., Ltd.). EFSA Journal 2022;20(6):7342, 20 pp. https://doi.org/10.2903/j.efsa.2022.7342

Abbreviations

CFU	colony forming unit
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed