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## Safety and efficacy of Probion forte<sup>®</sup> (*Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P) for chickens for fattening

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### Abstract

In 2017, the EFSA Panel on Additive and Products or Substances used in Animal Feed (FEEDAP) delivered a scientific opinion on the safety and efficacy of Probion Forte<sup>®</sup> (*Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P) as a feed additive for chickens for fattening. The two bacterial species are considered suitable for the qualified presumption of safety (QPS) approach to safety assessment provided that the identity of the strains is established and the lack of toxigenic potential and acquired antimicrobial resistance for antibiotics of human and veterinary importance are demonstrated. In that opinion, the FEEDAP Panel was unable to conclude on the safety and efficacy of the additive due to inadequacy of the data available. In the current dossier, the applicant submitted a new cytotoxicity study in support to the safety of the active agents, a new skin sensitisation study and three new efficacy trials performed with chickens for fattening. The FEEDAP Panel identified a limitation in the experimental design of the cytotoxicity study which did not allow excluding the possible toxigenic potential of the strains. One of the three efficacy trials was not considered due to the high mortality (including culling) reported in the control group. On the basis of the data available, the FEEDAP Panel could not conclude on the safety of Probion Forte<sup>®</sup> for the target species and for the consumer. Probion Forte<sup>®</sup> was not considered a skin sensitiser. The FEEDAP Panel could not conclude on the efficacy of Probion Forte<sup>®</sup> for chickens for fattening.

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## 1. Introduction

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

Regulation (EC) No 1831/2003<sup>1</sup> established 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, Agencja Inwestycyjno-Handlowa B&B dr ini. Zbigniew Borowski, is seeking a Community authorisation of *Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P as a feed additive to be used as a gut flora stabiliser for laying hens, chickens for fattening and chickens reared for laying. (Table 1)

**Table 1:** Description of the substances

<b>Category of additive</b>	Zootechnical additive
<b>Functional group of additive</b>	Gut flora stabilisers
<b>Description</b>	<i>Bacillus subtilis</i> KCCM 10941P and <i>Bacillus coagulans</i> KCCM11093P
<b>Target animal category</b>	Laying hens, chickens for fattening and chickens reared for laying
<b>Applicant</b>	Agencja Inwestycyjno – Handlowa B&B dr ini. Zbigniew Borowski
<b>Type of request</b>	New application

On 06 April 2017, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority (“Authority”), in its opinion, on the safety and efficacy of the product, could not conclude on the safety of *Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P as a feed additive for laying hens, chickens for fattening and chickens reared for laying due to the toxigenic potential.

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 17 October 2018.

In view of the above, the Commission asks the Authority to deliver a new opinion on *Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P as a feed additive for laying hens, chickens for fattening and chickens reared for laying based on the additional data submitted by the applicant.

### 1.2. Additional information

The EFSA Panel on Additive and Products or Substances used in Animal Feed (FEEDAP), in 2017, delivered a scientific opinion on the safety and efficacy of Probion Forte® (*Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P) as a feed additive for chickens for fattening (EFSA FEEDAP Panel, 2017). In that opinion, the FEEDAP Panel was unable to conclude on the safety and efficacy of the additive due to inadequacy of the data available.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on the data submitted by the applicant in the form of additional information<sup>2</sup> following a previous application on the same product.<sup>3</sup>

### 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of Probion Forte® (*Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P) is in line with the principles laid down in Regulation (EC) No 429/2008<sup>4</sup> and the relevant guidance documents: Guidance on the characterisation of microorganisms used as feed additives or as production strains (EFSA FEEDAP Panel, 2018a),

<sup>1</sup> 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.

<sup>2</sup> Dossier reference: FAD-2018-0076.

<sup>3</sup> Dossier reference: FAD-2014-0038.

<sup>4</sup> 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.

Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b).

### 3. Assessment

Probion Forte® is a preparation of spores of *B. subtilis* and *B. coagulans*. The additive is intended to be used as a zootechnical additive (functional group: gut flora stabiliser) in feed for chickens for fattening at the minimum recommended level of  $1 \times 10^8$  colony forming unit (CFU)/kg (equivalent to 500 mg/kg feedingstuffs) and a maximum level of  $2 \times 10^8$  CFU/kg feedingstuffs (equivalent to 1,000 mg/kg feedingstuffs).

In the original application, the applicant requested the use of the additive in chickens for fattening, chickens reared for laying and laying hens; however, during the course of that assessment, the applicant requested to limit the scope of the application to chickens for fattening only.

The applicant, in the current application, has submitted a new cytotoxicity study in support to the safety of the active agents, a new skin sensitisation study and three new efficacy trials performed with chickens for fattening. These studies aim at fulfilling the gaps identified in the previous assessment of the additive (EFSA FEEDAP Panel, 2017).

#### 3.1. Characterisation

The additive was fully characterised in the previous opinion in terms of physico-chemical properties, purity, technological properties and manufacturing process (EFSA FEEDAP Panel, 2017).

##### 3.1.1. Characterisation of the active agents

In its previous opinion, the FEEDAP Panel assessed a cytotoxicity study performed on fibroblast cells and concluded that, due to some limitations (i.e. unknown test item, inappropriate negative and positive controls, inappropriate cell line used), the study was not acceptable as a mean of excluding the toxigenic potential of the two strains (EFSA FEEDAP Panel, 2017).

In the current dossier, the applicant submitted a new cytotoxicity study using epithelial cell line (Vero cells) in order to demonstrate the absence of a toxigenic potential of the strains.<sup>5</sup> Cytotoxicity was measured by means of a lactate dehydrogenase (LDH) assay in accordance to the EFSA 'Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition' (EFSA FEEDAP Panel, 2014). However, the study was performed without an adequate positive control. This limitation in the experimental design does not allow the FEEDAP Panel to exclude the possible toxigenic potential of the strains.

#### 3.2. Safety

##### 3.2.1. Safety for the target species and for the consumer

The two bacterial species are considered suitable for the qualified presumption of safety (QPS) approach to safety assessment provided that the identity of the strains is established and the lack of toxigenic potential and acquired antimicrobial resistance for antibiotics of human and veterinary importance are demonstrated. In the previous opinion, the identity of the two active agents was established and the lack of acquired antimicrobials determinants confirmed. However, since the absence of toxigenic potential was not demonstrated, these strains did not comply with the QPS qualification to safety assessment. Consequently, they could not be presumed safe for the target species and consumers of products derived from animals treated with the additive without additional studies (EFSA FEEDAP Panel, 2017).

The applicant did not provide data to fill the gap identified in the previous opinion. Therefore, in the absence of evidence that the two strains are not toxigenic, the safety of the additive for the target species and for the consumer cannot be established.

<sup>5</sup> Technical dossier/cytotoxicity\_study.

### 3.2.2. Safety for the user

In its previous opinion, the FEEDAP Panel assessed an acute dermal irritation study and an acute eye irritation study and concluded that Probion Forte® was not a skin or eye irritant. In the absence on data, no conclusion could be drawn on the skin sensitisation potential. Moreover, the additive was considered a potential respiratory sensitiser. In the absence of evidence that the two active agents are not toxicogenic, Probion Forte® is considered as a respiratory hazard for those handling the additive (EFSA FEEDAP Panel, 2017).

In the current dossier, the applicant provided a new skin sensitisation study<sup>6</sup> performed in accordance to the OECD Guideline 406. Probion Forte® did not show any skin sensitisation potential.

### 3.2.3. Safety for the environment

The strains composing the additive belong to species which are ubiquitous soil saprophytes. Consequently, use of the strains in animal nutrition is not expected to measurably increase their numbers in the environment. Therefore, its use as a feed additive is considered safe for the environment (EFSA FEEDAP Panel, 2017).

## 3.3. Efficacy for chickens for fattening

In its previous opinion, the FEEDAP Panel assessed six trials in support of the efficacy of Probion Forte® when used in chickens for fattening (EFSA FEEDAP Panel, 2017). Due to some limitations in the experiments and to the incomplete reporting of some experimental details, the FEEDAP Panel was unable to conclude on the efficacy of the additive when used in chickens for fattening.

In the current dossier, the applicant submitted three new efficacy trials. However, one of the trials was not further considered due to the high mortality (including culling) in the control group (11%).

The two trials considered were done in the same trial site and followed the same trial design. In each trial, a total of 480 one-day-old male chickens for fattening (Ross 308) were distributed in 60 floor pens in groups of eight animals and allocated to three dietary treatments (20 replicates per treatment) for 42 days.<sup>7,8</sup> Three basal mash diets (starter, grower and finisher) based on soybean meal (SBM) 44% crude protein (CP), maize, wheat, rapeseed meal, lard and soy oil were either not supplemented (control) or supplemented with Probion Forte® to provide 0, 500 or 1,000 mg additive/kg feed, corresponding to 0, *Bacillus subtilis*  $0.58 \times 10^8$ /*Bacillus coagulans*  $0.56 \times 10^8$  CFU/kg feed and *Bacillus subtilis*  $1.17 \times 10^8$ /*Bacillus coagulans*  $1.12 \times 10^8$  CFU/kg, respectively.

Mortality and health status were monitored daily. Animals were weighed on days 0, 11, 21 and end (pen basis), feed intake was registered per pen and feed to gain ratio calculated. An analysis of variance (ANOVA) was done with the performance data (pen basis) and considering the treatment as the effect. Group means were compared with the Duncan test. Mortality was analysed using the Kruskal–Wallis method. The significance level was set at 0.05.

The results are presented in Table 2. Birds receiving the additive had a higher body weight compared to the control in the two trials, one at the lowest recommended dose and one at the highest dose. A significant better feed to gain ratio was observed compared to the control at the lowest recommended level in one of the trials (trial 1).

**Table 2:** Effects of Probion Forte® on the performance of chickens for fattening

Study No	Treatment groups		Total feed intake per animal (g)	Final body weight (g)	Feed to gain ratio	Mortality and culling % (n.)
	CFU/kg feed	mg/kg feed				
1	0	0	4,146	2,672 <sup>b</sup>	1.55 <sup>a</sup>	1.3 (2)
	$1 \times 10^8$	500	4,136	2,780 <sup>a</sup>	1.49 <sup>b</sup>	0.6 (1)
	$2 \times 10^8$	1,000	4,227	2,813 <sup>a</sup>	1.50 <sup>b</sup>	1.9 (3)

<sup>6</sup> Technical dossier/Skin Sensitization\_Study.

<sup>7</sup> Technical dossier/Probion-Forte\_Study1.

<sup>8</sup> Technical dossier/Probion-Forte\_Study3.

Study No	Treatment groups		Total feed intake per animal (g)	Final body weight (g)	Feed to gain ratio	Mortality and culling % (n.)
	CFU/kg feed	mg/kg feed				
2	0	0	3,766	2,433 <sup>b</sup>	1.55	0.65 (3)
	1 × 10 <sup>8</sup>	500	3,754	2,479 <sup>b</sup>	1.52	0.41 (2)
	2 × 10 <sup>8</sup>	1,000	3,778	2,590 <sup>a</sup>	1.46	0.82 (4)

CFU: colony forming unit.

<sup>a,b</sup>: Mean values within a column with a different superscript are significantly different  $p < 0.05$ .

### 3.3.1. Conclusions on efficacy for the target species

The FEEDAP Panel, in the absence of a third trial showing positive effects of the additive on the performance of the birds, cannot conclude on the efficacy of Probion Forte® in chickens for fattening.

## 4. Conclusions

The absence of toxigenic potential of the strains (*B. subtilis* KCCM 10941P and *B. coagulans* KCCM 11093P) has not been demonstrated, and therefore, the qualifications for the QPS approach were not met. On the basis of the data submitted by the applicant, the FEEDAP Panel cannot conclude on the safety of Probion Forte® for the target species and for the consumer.

The new data evaluated indicate that Probion Forte® is not a skin sensitiser. In the absence of evidence that the two active agents are not toxigenic, Probion Forte® is considered as a respiratory hazard for those handling the additive.

The FEEDAP Panel cannot conclude on the efficacy of Probion Forte® for chickens for fattening.

## Documentation provided to EFSA

- 1) *Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P. October 2018. Submitted by Agencja Inwestycyjno on the behalf of WooGene B&B Co.,Ltd.

## Chronology

Date	Event
23/10/2018	Dossier received by EFSA
30/11/2018	Reception mandate from the European Commission
26/02/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

## References

- EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2012. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. <https://doi.org/10.2903/j.efsa.2012.2539>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Guidance on the assessment of the toxigenic potential of Bacillus species used in animal nutrition. EFSA Journal 2014;12(5):3665, 10 pp. <https://doi.org/10.2903/j.efsa.2014.3665>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2017. Scientific Opinion on the safety and efficacy of Probion Forte (*Bacillus subtilis* KCCM 10941P and *Bacillus coagulans* KCCM 11093P) as a feed additive for chickens for fattening. EFSA Journal 2017;15(4):4759, 10 pp. <https://doi.org/10.2903/j.efsa.2017.4759>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2018a. Guidance on the characterization of microorganisms used as feed additives or as production organisms. EFSA Journal 2018;16(53):5206, 24 pp. <https://doi.org/10.2903/j.efsa.2018.5206>
- EFSA FEEDAP Panel (EFSA Panel on additives and products or substances used in animal feed), 2018b. Guidance on the assessment of the efficacy of feed additives. EFSA Journal 2018;16(5):5274, 25 pp. <https://doi.org/10.2903/j.efsa.2018.5274>

## Abbreviations

ANOVA	analysis of variance
CFU	colony forming unit
CP	crude protein
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
LDH	lactate dehydrogenase
SBM	soybean meal
QPS	qualified presumption of safety