REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment on the Safety and Efficacy of *Bacillus Velezensis* ATCC PTA-6737 (PB6) as a Feed Additive for Weaned Piglets, Weaned Minor Porcine Species, Sows and All Pig species. (RP1512)

Food Standards Agency[1], Food Standards Scotland[2]

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

Keywords: Regulated Products, Feed Additives, Safety Assessment

https://doi.org/10.46756/001c.129339

FSA Research and Evidence

An application was submitted to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in April 2022 from Kemin Europa N.V. ('the applicant') for the renewal and new use (extension of species) to all pig species of an additive containing *Bacillus velezensis* ATCC PTA-6737 under the category 'zootechnical', functional group 'gut flora stabiliser'. The additive is proposed to be used at a minimum content of 1 x 10^7 CFU/kg complete feedingstuff in piglets (weaned), minor porcine species (weaned) and in growing pigs, and at a minimum content of 1 x 10^8 CFU/kg complete feedingstuff in sows and minor reproductive porcine species.

To support the FSA and FSS in evaluating the dossier, the Advisory Committee on Animal Feedingstuffs (ACAF) were asked to review the dossier and the supplementary information from the applicant.

The FSA/FSS concluded, based on the ACAF's advice, that the additive was correctly identified and characterised, and that it is stable for 18 months at room temperature, for 6 months as part of a mineral premix and a finished mash feed, and for 3 months after pelleting when held at a maximum of 90°C for 45 seconds. Homogeneity was also demonstrated.

The ACAF concluded that the additive is presumed safe for the target species, the consumer and the environment due to its Qualified Presumption of Safety (QPS) status. The additive is not an irritant to skin or eyes but should be treated as a potential skin and respiratory sensitiser.

The ACAF concluded that the additive is efficacious in weaned piglets at a dose of 1 x 10^7 CFU/kg complete feed. The Committee also concluded that the additive has the potential to be efficacious in sows and minor reproductive species at a dose of 1 x 10^8 CFU/kg complete feed. The Committee could not accept the applicant's specific request for extrapolation from piglets to all growing pigs due to lack of evidence for efficacy when fed at the lower dose (1 x 10^7 CFU/kg) proposed in pigs.

However, given the presence of three significant piglet trials and three significant sow trials the Committee was satisfied that the studies provided supported efficacy in all pigs when the higher dose of 1 x 10^8 CFU/kg was utilised for pigs in the fattening phase.

The views of ACAF have been taken into account in the safety assessment which represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.

1. Introduction

The FSA and FSS have undertaken an assessment for a feed additive (PB6) containing *Bacillus velezensis* ATCC PTA-6737 (Kemin Europa N.V. in Toekomstlaan 42, Herentals, 2200, Belgium) under Assimilated Regulation (EC) No 1831/2003. The application sought a renewal authorisation for use in sows, weaned piglets and weaned minor porcine species, as well as an extension of use to all pig species. The additive falls under the category 'zootechnical additive', functional group 'gut flora stabiliser'.

To support the safety assessment by FSA and FSS, the ACAF provided advice to the FSA and FSS outlined in this document.

In line with Article 8 of 1831/2003, the assessment has considered and concluded that the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by EFSA for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

The dossier was evaluated by the Advisory Committee on Animal Feedingstuffs (ACAF) at its January 2024 meeting, after which a request for further information was communicated to the applicant. The applicant's response to this request and subsequent requests were evaluated by ACAF at its April and June 2024 meetings.

The views of ACAF have been taken into account in this safety assessment which represents the opinion of the FSA/FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended dose/intake
Bacillus velezensis ATCC PTA-6737 (PB6)	Feed additive	Zootechnical additive	1×10^7 – 1×10^8 CFU/kg complete feedingstuff

2. Assessment

2.1. Section II: Identity, characterisation and conditions of use

The active ingredient of PB6 are spores of *Bacillus velezensis* ATCC PTA-6737 at a minimum concentration of 8.0×10^{10} CFU spores per gram. PB6 is a dry powder consisting of 75 – 95% sodium bicarbonate, used as a carrier, and 5 – 25% dried ferment. The applicant provided data from five batches supporting the identification values outlined below (Table 2):

Table 2. Identity table for *Bacillus velezensis* ATCC PTA-6737 (PB6)

Composition	
Active substance: *Bacillus velezensis* ATCC PTA-6737	5-25 %
Carrier: Sodium bicarbonate	75-95 %
Appearance	
Free-flowing white powder	
Chemical-physical properties	
Dusting potential	1.045 – 1.540 g/m^3
Particle size distribution	< 100 µm – 97.47 – 99.85 % < 50 µm – 89.43 – 97.30 % < 10µm – 19.3 – 32.38 %
Bulk density	500 – 600 g/L
Impurities	
Arsenic	≤ 2 ppm
Lead	≤ 10 ppm
Cadmium	≤ 1 ppm
Mercury	≤ 0.1 ppm
Fluorine	<40 mg/kg
E. coli	Absent in 10 g
Salmonella spp.	Absent in 25 g
S. aureus	Absent in 10 g
Coliforms	Absent in 10 g
Yeast and moulds	< 10 CFU/g
Pesticides	< 10 ppb
Dioxin and dioxin-like PCBs	< 1.25 ppt TEQ

Composition	
Deoxynivalenol	< 900 ppb
Fumonisin	< 5,000 ppm
Aflatoxin B1	< 20 ppb
Ochratoxin A	< 50 ppb
Zearalenone	< 100 ppb

The applicant provided clarification regarding the presence of AMR genes, as well as the absence of plasmids, allowing the Committee to agree that the strain had been fully characterised using whole genome sequencing. Members were also able to conclude on the identity and characterisation of the additive after the applicant provided more recent data demonstrating batch-to-batch variation and impurity testing. Supporting documentation and certification relating to quality assurance and further information on Hazard Analysis and Critical Control Point (HACCP) principles, including critical control points, were also provided upon request.

Members noted a discrepancy in the dusting potential values given. Clarification provided by the applicant confirmed that the dusting potential values for this additive are quite high, as these values range from 1.045 to 1.540 g/m^3. The additive consists of particles of inhalable size (< 100 µm) and has a high proportion of particles of respirable size (< 10 µm).

The additive was shown to be stable for 12 months at 40°C and 6 months at 55°C. There were no statistically significant differences between the counts at the start of the study and after 18 months of storage at room temperature. PB6 was also demonstrated to be stable for 6 months when added to both a mineral premix and a finished mash feed, and remained stable for 3 months after pelleting at a maximum of 90°C for 45 seconds. Homogeneity in premixture and in feed samples was also demonstrated.

The Committee noted that the additive is authorised for use in weaned piglets at a minimum content of 1 x 10^7 CFU/kg complete feed and is intended for use in growing pigs at a minimum content of 1 x 10^7 CFU/kg complete feed, and in sows and minor reproductive species at 1 x 10^8 CFU/kg complete feed. The applicant was asked to clarify how they concluded on the dose for growing pigs, as efficacy trials were performed using different doses for weaned piglets and sows. In their response, the applicant explained that the efficacy data from piglets was extrapolated to growing pigs, in line with guidance, hence growing pigs would receive the same dose as piglets. Members could not accept the applicant's specific request for extrapolation from piglets to all growing pigs due to the lack of evidence for efficacy when fed at the lower dose (1 x 10^7 CFU/kg) proposed in piglets and considering the difference in physiological age between piglets and pigs for fattening. However, given the presence of

three significant piglet trials and three significant sow trials, the Committee acknowledged that the studies provided could provide supporting evidence of efficacy in all pigs when a minimum content of 1 x 10^8 CFU/kg was utilised for pigs in the fattening phase. The Committee decided on this higher dose of 1 x 10^8 CFU/kg for pigs for fattening due to physiological state (i.e. they are more mature animals), and an uncertainty in the additive's efficacy in older animals; therefore the higher dose was more appropriate to ensure efficacy in pigs for fattening. Consequently, the Committee concluded that the additive has the potential to be efficacious in all growing pigs, but use in pigs for fattening would be at the higher dose of 1 x 10^8 CFU/kg complete feed.

The proposed conditions of use of the additive are described in Table 3.

Table 3. Conditions of use of *Bacillus velezensis* ATCC PTA-6737 proposed by the applicant

Species or category of animal	Min. content in feed	Max. content in feed	Withdrawal period
Piglets (weaned) and minor porcine species (weaned)	1 x 10^7 CFU/kg of complete feedingstuff	-	-
Pigs for fattening	1 x 10^7 CFU/kg of complete feedingstuff	-	-
Sows and minor reproductive porcine species	1 x 10^8 CFU/kg of complete feedingstuff	-	
Other provisions and additional requirements for the labelling			
Specific conditions or restrictions for use	Store in a dry place in closed containers at room temperature. Stable to pelleting at max. 90°C for 45 seconds. May be used in compound feed containing the permitted coccidiostats: lasalocid, maduramycin, monensin, narasin, salinomycin, decoquinate, robenidine, diclazuril and narasin/nicarbazin		
Specific conditions or restrictions for handling	For user safety – wear personal protective equipment/face protection. Ensure adequate ventilation. Avoid contact with skin, eyes or clothing. Avoid dust formation.		

The ACAF concluded on a higher dose of 1 x 10^8 CFU/kg of complete feedingstuff as being potentially efficacious for use in pigs for fattening. This is discussed further in Section IV.

2.1.1. Conclusions on Section II

The ACAF concluded that the additive was fully identified and characterised. Members were able to conclude positively on the stability and homogeneity of the additive.

Regarding conditions of use, the ACAF were able to conclude positively on those outlined by the applicant, however use in pigs for fattening would be at the higher dose of 1 x 10^8 CFU/kg of complete feedingstuff. No further concerns were raised for Section II of the dossier.

2.2. Section III: Safety

2.2.1. Safety for the target species

No evaluation of the safety for the target species was required given the Qualified Presumption of Safety (QPS) status confirmation of *Bacillus velezensis* ATCC PTA-6737. The additive is therefore presumed safe for the target species.

2.2.2. Safety for the consumer

No evaluation of the safety for the consumer was required given the QPS status confirmation of *Bacillus velezensis* ATCC PTA-6737; however, the applicant provided additional data relating to safety for the consumer to support its QPS status. The applicant provided the following tests: an acute oral toxicity test, an erythrocyte micronucleus assay in mice and a 28-day repeat dose oral toxicity study using doses of up to 1,000 mg PB6/kg bw/day. The Committee concluded that the toxicological studies provided demonstrated no adverse effects and that the additive could be considered safe for the consumer.

2.2.3. Safety for the user

The applicant presented the following tests to evaluate the safety of the additive for the user/worker:

- Acute inhalation test, following OECD protocol 403;
- Acute dermal irritation/corrosion study, following OECD protocol 404;
- Acute eye irritation/corrosion study, following OECD protocol 405.

The Committee concluded that PB6 is not irritant to skin or eyes, but that it should be treated as a potential skin sensitiser as the applicant has provided insufficient evidence proving otherwise. The ACAF also concluded that as a microbial product PB6 is regarded as a potential respiratory sensitiser and it could deposit in the respiratory tract of exposed workers; therefore inhalation should be avoided.

2.2.4. Safety for the environment

No evaluation of the safety for the environment was required given the QPS status confirmation of *Bacillus velezensis* ATCC PTA-6737. The additive is therefore presumed safe for the environment.

2.2.5. Conclusions on safety

The ACAF concluded that the additive is presumed safe for the target animals, the consumers, and the environment.

The additive is not irritant to skin or eyes but should be treated as a potential skin and respiratory sensitiser.

2.3. Section IV: Efficacy

The applicant presented four efficacy studies in weaned piglets and four studies in sows to allow for extrapolation to all pig species at all growing phases. The applicant concluded that supplementation with *Bacillus velezensis* ATCC PTA-6737 (PB6) in the diet of growing piglets at dosages of 1×10^7 CFU/kg and 5×10^7 CFU/kg results in improvements in zootechnical parameters. The applicant also concluded that supplementation with the additive in sow diets at a dosage of 1×10^8 CFU/kg significantly improves the performance of sows and their piglets.

The Committee assessed the efficacy trials provided for weaned piglets and concluded that the additive is efficacious in weaned piglets at a dose of 1×10^7 CFU/kg complete feed. The Committee noted several inconsistencies within the sow trials and only two of the trials appeared to demonstrate efficacy; however, three trials are needed to provide a conclusion. Further clarification was therefore requested regarding efficacy in sows and reproductive stages of minor porcine species and on the proposed doses for use in pigs for fattening. An additional trial could not be provided by the applicant for use in sows, and therefore the ACAF concluded that the additive has the potential to be efficacious in sows at a dose of 1×10^8 CFU/kg complete feed.

After assessing the information provided the Committee also concluded that the data provided was suitable for extrapolation to all pigs in line with the guidance, as the applicant had provided sufficient studies for weaned piglets and sows. The Committee therefore concluded that the additive is efficacious in weaned piglets at a dose of 1×10^7 CFU/kg complete feed and has the potential to be efficacious in sows and pigs for fattening at a dose of 1×10^8 CFU/kg complete feed.

2.3.1. Conclusions on efficacy

The ACAF concluded that *Bacillus velezensis* ATCC PTA-6737 is efficacious in weaned piglets at a dose of 1 x 10^7 CFU/kg complete feed and has the potential to be efficacious in sows and pigs for fattening at a dose of 1 x 10^8 CFU/kg complete feed.

3. Analytical methods evaluation

Conclusions on the analytical methods are presented here as an extract from the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of the Analysis for *Bacillus subtilis* ATCC PTA-6737 (EURL 2009):

> "For the enumeration of *Bacillus subtilis* ATCC PTA-6737 in the feed additive, premixtures and feedingstuffs, the applicant proposes the draft CEN method – prEN 15784:2008 E – an internationally recognised spread plate method. This method was ring-trial validated using the premixtures and feedingstuffs samples containing *Bacillus subtilis* spores. The performance characteristics of the draft CEN method reported after logarithmic transformation of measured values (CFU) are:
>
> - For the premixtures: (1) a standard deviation for repeatability (s_r) of 0.09 $\log_{10}$ and (2) a standard deviation for between-laboratory reproducibility (s_R) of 0.32 $\log_{10}$.
> - For the feedingstuffs: (1) a s_r of 0.07 $\log_{10}$ and (2) a s_R of 0.35 $\log_{10}$.
>
> The applicant used the above mentioned spread plate method to analyse the various matrices containing *Bacillus subtilis* ATCC PTA-6767 spores and reported the following results: (a) 1 x 10^9 to 1.5 x 10^{11} CFU/g of feed additive; (b) 1 x 10^7 to 1.5 x 10^9 CFU/kg for premixtures and (c) 1 x 10^7 to 1.5 x 10^8 for feedingstuffs. The results obtained for feed additives and premixtures are considered acceptable; this method is therefore recommended for official controls for the feed additives and premixtures in the frame of the authorisation.
>
> As regards feedingstuffs, the CRL notes that the limit of quantification reported by the applicant upon request (LOQ = 1 x 10^7 CFU/kg feedingstuffs) is identical to the minimum dose proposed and is below the LOQ reported in the draft CEN method (2 x 10^7 CFU/kg). On the basis of the available

> information, the draft CEN method is recommended for official control of the feedingstuffs containing *Bacillus subtilis* PB6 at the dosages above the LOQ reported by CEN. Below 2 x 10^7 CFU/kg the CRL is not able to conclude on the suitability of this method for official control purposes.
>
> Molecular methods were used by the applicant for identification of the active agent. For official controls pulsed field gel electrophoresis (PFGE), a generally recognised standard methodology for microbial identification, is recommended.
>
> Further testing or validation is not considered necessary."

FSA/FSS accepts the EURL analytical method evaluation reports. FSA/FSS determined the analytical methods proposed as appropriate for official controls for this feed additive.

4. Conclusions

The ACAF concluded that the additive was fully identified and characterised. Members concluded positively on the stability and homogeneity of the additive.

The additive is presumed safe for the target animals, the consumer and the environment. It is not irritant to skin or eyes but should be treated as a potential skin and respiratory sensitiser.

The ACAF concluded that *Bacillus velezensis* ATCC PTA-6737 is efficacious in weaned piglets at a dose of 1 x 10^7 CFU/kg complete feed. The Committee also concluded that the additive has the potential to be efficacious in sows at a dose of 1 x 10^8 CFU/kg complete feed. As suitable efficacy trials were submitted for both sows and piglets, the data provided could be extrapolated to all pigs in line with the guidance, however the Committee noted that when fed to pigs during the fattening phase the higher dose of 1 x 10^8 CFU/kg should be utilised.

Abbreviations

Abbreviation	Definition
ACAF	Advisory Committee for Animal Feedingstuffs
CEN	Comité de Normalizaition
CFU	Colony forming units
CRL	Community Reference Laboratory
EC	Euorpean Commission
EURA-FA	European Reference Laboratory for Feed Additives
FSA	Food Standards Agency

Abbreviation	Definition
FSS	Food Standards Scotland
HACCP	Hazard Analysis and Critical Control Points
HPLC	High pressure liquid chromatography
LOQ	Limit of quantification
PFGE	Pulsed field gel electrophoresis
QPS	Qualified Presumption of Safety
S_r	Standard deviation for repeatability
s_R	Standard deviation for between-laboratory reproducibility

Acknowledgements

With thanks to the members of the ACAF during the course of the assessment, who were: Professor Nicholas Jonsson, Martin Briggs, Professor Emily Burton, Professor Katrina Campbell, Professor Matthew Fisher, Hannah Kane, Susan MacDonald, Dr. Oonagh Markey, Christine McAlinden, Dr. Donald Morrison, Derek Renshaw, Dr. Michael Salter, Dr. Adam Smith, Christel Wake, Dr. Helen Warren and Professor Nick Wheelhouse.

Published: February 28, 2025 GMT.

References

EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. https://www.legislation.gov.uk/eur/2003/1831/contents

EURL-FA (European Reference Laboratory for Feed Additives). (2009). *Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Bacillus subtilis ATCC PTA-6737*.