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Safety and efficacy of *Bacillus subtilis* PB6 (*Bacillus subtilis* ATCC PTA-6737) as a feed additive for sows

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria De Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Rosella Brozzi and Maria Saarela

Abstract

Bacillus subtilis PB6 is a feed additive based on viable spores of a strain of *Bacillus subtilis*. EFSA has already issued several opinions on the safety and efficacy of the additive when used in the feed for a number of avian and porcine species/categories. The applicant is now seeking authorisation for use in sows in order to have benefits in piglets. In the course of previous assessments, no evidence of a toxigenic potential or resistance to relevant antibiotics was found. Consequently, the strain of *B. subtilis* in the additive, following the qualified presumption of safety approach to safety assessment, is presumed safe for target animals including sows and their offspring, consumers and the environment. In a previous assessment, it was also concluded that the additive is not a skin/eye irritant or a skin sensitiser and that there were no concerns on respiratory sensitisation. The use of the additive with feed for sows is considered unlikely to introduce hazards for users of the product not already considered. Five studies are described in which groups of sows given the additive for a minimum period from the last 3 weeks of pregnancy, throughout farrowing and lactation, until weaning of piglets were compared with a control group. In only two of five studies, there was a significant beneficial effect seen in terms of piglet performance. However, when data from the four similar were pooled and analysed, significant increases in weaned weight and average daily gain were indicated for piglets from sows given the additive. Consequently, the FEEDAP Panel concludes that *Bacillus subtilis* PB6 when added to diets of sows from 3 weeks before parturition until weaning of piglets at a dose of 1×10^8 CFU/kg complete feed has the potential to improve the growth of piglets from birth to weaning.

Keywords: *Bacillus subtilis* PB6, *Bacillus subtilis* ATCC PTA-6737, QPS, efficacy, sows, piglets, growth

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Marta López Alonso, Secundino López Puente, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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Table of contents

1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Kemin Europa N.V.[2] for authorisation of the product *Bacillus subtilis* PB6 (*Bacillus subtilis* ATCC PTA-6737), when used as a feed additive for sows (category: zootechnical additive; functional group: gut flora stabiliser).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossiers in support of these applications. The particulars and documents in support of the application were considered valid by EFSA as of 7 October 2014.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product *Bacillus subtilis* PB6 (*Bacillus subtilis* ATCC PTA-6737), when used under the proposed conditions of use (see Section 3.1.1).

1.2. Additional information

EFSA has issued several opinions on the safety and efficacy of *Bacillus subtilis* PB6 including its use with chickens for fattening (EFSA FEEDAP Panel, 2009), chickens reared for laying, ducks for fattening, quails, pheasants, partridges, guinea fowl, pigeons, geese for fattening and ostriches (EFSA FEEDAP Panel, 2011a), weaned piglets and weaned minor porcine species (EFSA FEEDAP Panel, 2012a), turkeys for fattening and turkeys reared for breeding (EFSA FEEDAP Panel, 2013) and one for laying hens, other minor laying poultry birds (EFSA FEEDAP Panel, 2015). An opinion on the compatibility of *Bacillus subtilis* PB6 with coccidiostats was also published in 2010 (EFSA FEEDAP Panel, 2010).

This product is currently authorised for use as a feed additive in diets for chickens for fattening,[3] chickens reared for laying, ducks for fattening, quails, pheasants, partridges, guinea fowl, pigeons, geese for fattening, ostriches,[4] turkeys for fattening and reared for breeding,[5] for weaned piglets and weaned Suidae other than *Sus scrofa domesticus*[6] and for laying hens and minor poultry species for laying.[7]

[1] 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.
[2] Kemin Europa N.V., Toekomstlaan 42, 2200 Herentals, Belgium.
[3] Commission Regulation (EU) No 107/2010 of 8 February 2010 concerning the authorisation of *Bacillus subtilis* ATCC PTA-6737 as a feed additive for chickens for fattening (holder of authorisation Kemin Europa NV). OJ L 36, 9.2.2010, p. 1.
[4] Commission Implementing Regulation (EU) No 885/2011 of 5 September 2011 concerning the authorisation of *Bacillus subtilis* (ATCC PTA-6737) as a feed additive for chickens reared for laying, ducks for fattening, quails, pheasants, partridges, guinea fowl, pigeons, geese for fattening and ostriches (holder of authorisation Kemin Europa N.V.). OJ L 229, 6.9.2011, p. 3.
[5] Commission Implementing Regulation (EU) No 787/2013 of 16 August 2013 concerning the authorisation of a preparation of *Bacillus subtilis* (ATCC PTA-6737) as a feed additive for turkeys for fattening and turkeys reared for breeding (holder of authorisation Kemin Europa N.V.). OJ L 220, 17.8.2013, p. 15.
[6] Commission Implementing Regulation (EU) No 306/2013 of 2 April 2013 concerning the authorisation of a preparation of *Bacillus subtilis* (ATCC PTA-6737) for weaned piglets and weaned Suidae other than *Sus scrofa domesticus*. OJ L 91, 3.4.2013, p. 5.
[7] Commission Implementing Regulation (EU) 2015/1020 of 29 June 2015 concerning the authorisation of the preparation of *Bacillus subtilis* (ATCC PTA-6737) as a feed additive for laying hens and minor poultry species for laying. OJ L 163, 30.6.2015, p. 22.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of *Bacillus subtilis* PB6 (*Bacillus subtilis* ATCC PTA-6737) as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[9] and the applicable EFSA guidance documents.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Bacillus subtilis* PB6 is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012c) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b).

3. Assessment

The additive is a preparation containing viable spores of a strain of *Bacillus subtilis*. It is intended to be authorised as a zootechnical additive (functional group: gut flora stabilisers) in diets for sows to have benefits in piglets.

3.1. Characterisation

The additive is a preparation of viable spores of *Bacillus subtilis* ATCC PTA-6737 at a minimum declared concentration of 1×10^{10} CFU/g additive. It has the same formulation and method of manufacture as that considered in previous applications. Thus, the data pertaining to impurities, physical properties and shelf life still apply. Although no specific data on stability in feed for sows or ability to mix in such feed were provided, the data on stability in premixes, mash and pelleted feed for poultry and piglets are considered sufficient given the overall similarity in feed formulation.

3.1.1. Conditions of use

The product is intended for use in feed for sows at a dose of 1×10^8 CFU/kg complete feedingstuffs in order to have benefits in piglets. The recommended minimum duration of application is two weeks before parturition to the end of the weaning period.

3.2. Safety

The species *B. subtilis* is considered by EFSA to be suitable for the Qualified Presumption of Safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel, 2013). This approach requires the identity of the strain to be conclusively established, evidence that the strain is not toxigenic and that it does not show resistance to antibiotics of human and veterinary importance. EFSA considered these issues in its opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for chickens for fattening (EFSA FEEDAP Panel, 2009) using the guidance applicable at the time and concluded that the strain could be presumed safe for the target species, consumers and the environment. Subsequently, EFSA introduced new guidance on the determination of antibiotic susceptibility (EFSA FEEDAP Panel, 2012b) and assessing the toxigenic potential of *Bacillus* species (EFSA FEEDAP Panel, 2014). These issues were reconsidered taking account of the latest guidance in the context of an Opinion on an extension of use for laying hens and minor laying poultry birds (EFSA FEEDAP Panel, 2015). The suitability of the *Bacillus subtilis* PB6 strain to qualify for QPS safety

[8] FEED dossier reference: FAD-2014-0030.

[9] 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.

[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2008-0039.pdf

assessment was verified and the conclusions on the safety for target species, consumers and the environment confirmed. The FEEDAP Panel is unaware of any new data which would lead it to revise its conclusions.

In the course of the 2009 assessment for use with chicken for fattening, safety for users was also considered. The FEEDAP Panel concluded in that instance that *Bacillus subtilis* PB6 is not a skin/eye irritant, does not induce skin sensitisation and that data on the dusting potential of the additive do not give concerns of sensitisation via respiratory route. In the view of the FEEDAP Panel, use in diets for sows will not introduce hazards for users of the additive not already considered.

3.3. Efficacy

3.3.1. Individual trials

Data are presented from five trials performed in three different Member States in which the additive was added to the feed of sows during the reproductive cycle. A sixth study could not be considered because of the significantly higher number of piglets in the control group compared to the treatment group. Fewer piglets allows greater individual access to milk and potentially better growth consequently, the observed difference in piglet weights at weaning in this study could not be ascribed to the treatment alone with any degree of confidence.

During the course of the application, the statistical analysis of the raw data relating to piglet performance was repeated by an independent organisation. This re-analysis took into account that all piglets weaned by the same sow are correlated, and therefore cannot be treated as independent observations. Thus, the analysis of variance used the sow as the experimental unit rather than the piglet. All paired comparisons between the treatments were performed using Tukey's HSD test. The available denominator degrees of freedom for the test were approximated using the Kenward–Rodgers method and to check the normality assumption of the residuals and a QQ-plot was constructed for the residuals of each response.

The first trial[11] was designed as a dose–response study to determine the most effective treatment. A total of 81 sows (Hypor) were distributed to four experimental groups, giving regard to parity. The first group (15 sows) given only the basal diet (wheat/barley based in pelleted form) acted as controls while in the second (23 sows), third (22 sows) and fourth groups (21 sows), diets were supplemented with the additive at 1×10^7, 5×10^7 and 1×10^8 CFU/kg feed, respectively. The additive was provided over one full cycle in feed for sows (i.e. from insemination until weaning at approximately 29 days). The intended dose was confirmed by analysis of the feeds. Sows were monitored for feed intake, general health, weight changes and reproductive performance (total, live and stillborn piglets), and piglets for weight at birth and at weaning. From this, the average daily gain (ADG) of the piglets was calculated.

The second[12] study involved only two experimental groups, a control group and a test group in which the basal diet was supplemented with the additive at 1×10^8 CFU/kg feed (confirmed by analysis). In this study, 49 sows (Large White x Landrace) were distributed between the two groups with sow parity balanced across treatments. The test diets (wheat/barley/soybean in pelleted form) were fed in the last three weeks of pregnancy (beginning on day 90 after service), and continued throughout farrowing and lactation until piglets were weaned at approximately 28 days of age. Health and performance parameters were measured as for the first study. In addition, backfat thickness measurements in sows were made before and after farrowing and when piglets were weaned.

Studies 3[13] and 4[14] followed a similar design and were made at the same location. Both used Hypor sows and allocation was to the same two experimental groups, giving regard to parity and weight. In study 3, the control group consisted of 10 sows and the experimental group 12 sows and in study 4 both groups consisted of 12 sows. The dose 1×10^8 CFU/kg feed in all diets was confirmed by analysis of the treated feeds which were wheat/barley based and pelleted. The test diets were fed in the last three weeks of pregnancy (beginning on day 90 after service), and continued throughout farrowing and lactation until piglets were weaned at 28 days of age in study 3 and approximately 21 days of age in study 4. Health and performance parameters were measured as for the first study.

[11] Technical dossier/Section IV/Annexes IV.37-40 and Supplementary information May 2016/Annexes 8-9.
[12] Technical dossier/Section IV/Annexes IV.41-43 and Supplementary information May 2016/Annexes 10-11.
[13] Technical dossier/Supplementary information May 2016/Annexes 15-17.
[14] Technical dossier/Supplementary information May 2016/Annexes 18-20.

The final study[15] reported also followed the design of the earlier studies 2, 3 and 4. A total of 80 sows (described as Synthetic meat line 990) were allocated to one of the two experimental groups. Sows were fed cereal diets in mash form alone or the same diets supplemented with 1×10^8 CFU/kg (confirmed by analysis). The test diets were fed in the last 3 weeks of pregnancy (beginning on day 90 after service), and continued throughout farrowing and lactation until piglets were weaned at 28 days of age. In addition to the health and performance parameters measured in previous studies, backfat thickness and muscle depth was also recorded at the start of the study, after farrowing and at weaning.

The results of the five studies are summarised in Table 1.

Table 1: Summary of results of five efficacy studies with *Bacillus subtilis* PB6 in sows

Trial no	*Bacillus subtilis* PB6 (CFU/kg feed)	Number of sows	Piglets born alive (n)	Piglet weight at birth (kg)	No piglets at weaning (n)	Piglet weight at weaning (kg)	Average daily gain during suckling (kg)
1	0	15	13.3	1.54	10.8	7.15	0.20
	1×10^7	23	12.5	1.49	9.3	7.62	0.21
	5×10^7	22	14.0	1.53	11.1	7.98	0.23
	1×10^8	21	13.2	1.58	10.7	8.05	0.22
2	0	25	12.5	1.50	11.3	8.26	0.27
	1×10^8	24	12.2	1.46	10.9	8.97*	0.28
3	0	10	12.0	1.63	9.7	6.75	0.19
	1×10^8	12	13.3	1.33*	10.9	7.97*	0.22*
4	0	12	13.7	1.53	n.r.	5.82	0.23
	1×10^8	12	13.0	1.50	n.r.	6.39	0.25
5	0	40	11.3	1.62	9.8	6.92	0.19
	1×10^8	40	11.2	1.59	9.8	6.85	0.19

CFU: colony forming unit; n.r. not reported.
*: Significantly different from the control value at $p < 0.05$.

In study 1, stillborns accounted for an average of 11% of total births with deaths during the suckling adding a further 2%. However, mortality was not treatment related. In this study, there were no significant improvements in performance characteristics detected for *Bacillus subtilis* PB6 treated sows (weight change, litter size (total born, born alive, stillbirths), number of piglets surviving to weaning or weight at weaning). None of the sows in study 2 died and no treatment-related effects were seen for piglet mortality. Treated sows better retained weight over the period of the study compared to control sows (sows given diets without the additive lost an average of 0.29 kg/day while those given the additive gained 0.05 kg/day). A significant effect of treatment on the weight of piglets was seen at weaning, with the treated group having a significantly higher mean body weight than that of the control group. In study 3, despite the significantly lower birth weight of the piglets from treated sows, their weight at weaning was significantly higher than those from the control group and the ADG during suckling was significantly better. In studies 4 and 5, no significant effects were seen for any of the measured parameters.

Overall, although there was a numerical trend towards increased weaned weight and ADG in treated piglets compared to those in the control group in four studies, this reached significance in only two (study 2 for weight at weaning, and study 3 for weight at weaning and ADG). Accordingly, an analysis combining data from the four of the five individual studies was made.

3.3.2. Analysis of pooled data

Data from four of the five trials (studies 2–5) were tested for homogeneity, pooled when appropriate and analysed. Trial 1 was excluded on the basis of the difference in design compared to the other four trials. In order to test the homogeneity of the remaining data, four homogeneity tests (O'Brien, Brown-Forsythe, Levene and Bartlett) were performed, with the intention of alleviating any bias that may be shown by individual statistical tests. Using these tests, homogeneity was

[15] Technical dossier/Supplementary information May 2016/Annexes 23-25.

demonstrated for the parameters related to piglet mortality and growth. The effects of the study and the treatment were included in the model as well as their interaction. No interactions between treatment and study were found. The sow was considered as the experimental unit.

The results of the analysis of pooled data showed no significant treatment effects for piglet mortality at birth, or from birth until weaning. However, the analysis did show a significant increase in weight of piglets at weaning (7.04 vs 7.64 kg, $p = 0.003$) in the treatment group compared to the control and a corresponding improvement in ADG (0.22 vs 0.24 kg, $p = 0.022$).

3.3.3. Conclusion on efficacy

In two of five studies, at least one significant beneficial effect was seen in terms of piglet performance (weight at weaning, ADG). This alone is considered by the Panel as insufficient evidence of efficacy. However, when data from the four studies which shared a similar design were pooled and analysed, significant increases in weaned weight and ADG of piglets from the treated group were indicated. Consequently, the FEEDAP Panel concludes that the additive *Bacillus subtilis* PB6 when added to diets of sows at a dose of 1×10^8 CFU/kg complete feed has the potential to improve the growth of piglets from birth to weaning.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[16] and Good Manufacturing Practice.

4. Conclusions

The active agent fulfils the requirements of the QPS approach to the assessment of safety and, when introduced into diets for sows, can be presumed safe for the sow and its offspring, for consumers of products derived from pigs and for the environment.

Bacillus subtilis PB6 is not a skin/eye irritant or a skin sensitiser. Data on the dusting potential of the additive do not give concerns of sensitisation via respiratory route. The additional use of the additive with feed for sows is considered unlikely to introduce hazards for users of the product not already considered in previous assessments of *Bacillus subtilis* PB6.

On the basis of the results of the analysis of data pooled from four studies which shared a similar design, the FEEDAP Panel concludes that the additive *Bacillus subtilis* PB6 when added to diets of sows from 3 weeks before parturition until weaning of piglets at a dose of 1×10^8 CFU/kg complete feed has the potential to improve the growth of piglets from birth to weaning.

Documentation provided to EFSA

1) *Bacillus subtilis* ATCC PTA-6737. New use: Sows, in order to have benefit in piglets. August 2014. Submitted by Kemin Europa N.V.
2) *Bacillus subtilis* ATCC PTA-6737. New use: Sows, in order to have benefit in piglets. Supplementary information. February 2015. Submitted by Kemin Europa N.V.
3) *Bacillus subtilis* ATCC PTA-6737. New use: Sows, in order to have benefit in piglets. Supplementary information. May 2016. Submitted by Kemin Europa N.V.
4) *Bacillus subtilis* ATCC PTA-6737. New use: Sows, in order to have benefit in piglets. Supplementary information. February 2017. Submitted by Kemin Europa N.V.
5) Comments from Member States.

[16] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance for the preparation of dossiers for zootechnical additives. EFSA Journal 2012;10(1):2536, 19 pp. https://doi.org/10.2903/j.efsa.2012.2536

EFSA FEEDAP Panel (Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific Opinion on the safety and efficacy of *Bacillus subtilis* PB6 (*Bacillus subtilis*) as a feed additive for turkeys for fattening and turkeys reared for breeding. EFSA Journal 2013;11(4):3176, 10 pp. https://doi.org/10.2903/j.efsa.2013.3176

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), 2015. Scientific Opinion on the safety and efficacy of *Bacillus subtilis* PB6 as a feed additive for laying hens and minor poultry species for laying. EFSA Journal 2015;13(1):3970, 10 pp. https://doi.org/10.2903/j.efsa.2015.3970

Abbreviations

ADG	average daily gain
CFU	colony forming unit
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
HSD	honest significant difference
Q-Q	quantile-quantile
QPS	Qualified Presumption of Safety