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Safety and efficacy of a feed additive consisting of *Bacillus velezensis* NITE BP-01844 (BA-KING®) for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and all avian species for fattening, or rearing to slaughter or point of lay including non-food producing species (Toa Biopharma Co., Ltd.)

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of BA-KING® *Bacillus velezensis* when used as a feed additive for chickens for fattening, turkeys for fattening, chickens reared for laying, turkeys reared for breeding and all avian species for fattening, or rearing to slaughter or point of lay including non-food producing species. The product under assessment is based on viable spores of a strain identified as *B. velezensis*, which is considered suitable for the qualified presumption of safety (QPS) approach to safety assessment. The identity of the active agent was established, and it does not harbour acquired antimicrobial resistance genes, lacks toxigenic potential and does not have the capacity to produce aminoglycosides. Following the QPS approach to safety assessment, *B. velezensis* NITE BP-01844 is presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. Since no concerns are expected from the other components of the additive, BA-KING® is also considered safe for the target species, consumers of products derived from animals fed the additive and the environment. BA-KING® is not irritant to skin but is potentially irritant to eyes. In addition, should be considered a skin and respiratory sensitiser. The Panel is not in the position to conclude on the efficacy of BA-KING® for the target species.

Keywords: zootechnical additive, gut flora stabilisers, BA-KING®, *Bacillus velezensis* NITE BP-01844, QPS, safety, efficacy

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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 Toa Biopharma Co., Ltd., Japan, represented in the European Union by Toa Biopharma Co., Ltd. Europe Representative Office,[2] for authorisation of the product BA-KING® (*Bacillus velezensis*[3] NITE BP-01844), when used as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and all avian species for fattening, or rearing to slaughter or point of lay including non-food producing species (category: zootechnical additives; functional group: gut flora stabilisers).

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). The particulars and documents in support of the application were considered valid by EFSA as of 19 October 2020.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to 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 BA-KING® (*Bacillus velezensis* NITE BP-01844), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive BA-KING® is a preparation containing viable spores of *B. velezensis* NITE BP-01844, which has not been previously authorised 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 a technical dossier[4] in support of the authorisation request for the use of BA-KING® as a feed additive.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed. The Executive Summary of the EURL report can be found in Annex A.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of BA-KING® is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a),

[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] Toa Biopharma Co., Ltd., Japan represented by Toa Biopharma Co., Ltd., Europe Representative, Pl. Ausias March 1, 4th Floor, D10, ES-08195, St. Cugat del Vallès, Spain.
[3] Originally designated as *Bacillus amyloliquefaciens* ssp. *plantarum*.
[4] FEED dossier reference: FAD-2020-0049.
[5] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2020-0049_en
[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.

Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

BA-KING® is a preparation of viable spores of a single strain of *B. velezensis* (NITE BP-01844) intended for use as a zootechnical additive (functional group: gut flora stabilisers) in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and all avian species for fattening, or rearing to slaughter or point of lay including non-food producing species.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The active agent of BA-KING® is a strain originally isolated from healthy cattle and deposited at the Japanese National Institute of Technology and Evaluation Patent Microorganisms Depositary with the accession number NITE BP-01844.[7] The strain has not been genetically modified.

The taxonomic identification of the active agent NITE BP-01844 as *B. velezensis* was confirmed [redacted]

The toxigenic potential of *B. velezensis* NITE BP-01844 was assessed according to the Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018a).[9] Based on release of lactate dehydrogenase assay on Vero cells as a biomarker for cytotoxicity, the strain is considered to be non-cytotoxic.

The susceptibility of the strain to the antimicrobials recommended by the FEEDAP Panel was tested by agar dilution method following the Clinical and Laboratory Standards Institute (CLSI) standards.[10] All the minimum inhibitory concentration (MIC) values fell below the corresponding cut-off values. Therefore, *B. velezensis* NITE BP-01844 is susceptible to the relevant antibiotics.

[redacted]

To exclude the capacity of the active agent to produce aminoglycosides, culture supernatants from three batches were tested by the agar well diffusion method against reference strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633). No inhibition was observed, denoting the lack of antimicrobial production, including aminoglycosides.[12] [redacted]

3.1.2. Characterisation of the additive

BA-KING® is intended to be marketed in two formulations: one with defatted rice bran for use in feed and one with maltodextrin for use in water.

Viable spores of *B. velezensis* NITE BP-01844 are obtained [redacted] in order to reach the

[7] Technical dossier/Section II/Annex.II.2.1.2a.
[8] Technical dossier/Section II/Annex II.2.1.2.e and Annex II.2.1.2.f.
[9] Technical dossier/Section II/Annex II.2.2.2.b.
[10] Technical dossier/Section II/Annex II.2.2.2.c.
[11] Technical dossier/Section II/Annex II.2.1.2.f.
[12] Technical dossier/Section II/Annex II.2.1.2.d.
[13] Technical dossier/Section II/Annex II_3_1.

minimum guaranteed concentration of viable spores of 1×10^8 CFU/g of additive.[14] Compliance with this specification was demonstrated in six batches of the formulation with defatted rice bran (mean count 1.06×10^8 CFU/g, range 9.40×10^7 –1.1×10^8 CFU/g)[15] and in four pilot batches of the formulation with maltodextrin (mean count 6.28×10^{10} CFU/g, range 6.0×10^{10} –6.8×10^{10} CFU/g).[16]

According to the applicant, no antimicrobial substances are used during the manufacturing of the additive.[17]

Three batches of the formulation in defatted rice bran of the additive were analysed for the presence of chemical contaminants.[18] Values for dioxins, the sum of dioxins and dioxin-like PCBs, and non-dioxin-like PCBs were 0.077 ng WHO-TEQ/kg, 0.083-0.085 ng WHO-TEQ/kg and 0.052–0.093 μg/kg, respectively. Values for lead, mercury, arsenic and mycotoxins were below the respective limits of quantification (LOQs),[19] while cadmium ranged from 0.083 to 0.085 mg/kg (LOQ 0.05 mg/kg). The same batches were analysed for microbial contamination.[20] Results for the analyses were below the limit of detection (LOD) for *Salmonella* spp. (no detection in 25 g), total *Enterobacteriaceae* (< 10 CFU/g), presumptive *Bacillus cereus* (< 10 CFU/g), yeasts and filamentous fungi (< 10 CFU/g).

Four batches of the formulation in maltodextrin of the additive were analysed for chemical, excluding mycotoxins, and microbiological contaminants.[21] Lead and cadmium were detected only in two batches (0.07–0.12 mg Pb/kg and 0.013–0.054 mg Cd/kg), whereas arsenic and mercury were detected in only one batch (0.038 mg As/kg and 0.03 mg Hg/kg). Specifications are set for *Salmonella* spp. (no detection in 25 g), total *Enterobacteriaceae* (< 100 CFU/g), total coliforms (< 100 CFU/g), coagulase-positive staphylococci (including *S. aureus*) (< 10 CFU/g), presumptive *B. cereus* (< 100 CFU/g), yeasts and filamentous fungi (< 100 CFU/g), and the results for these parameters of the above-mentioned batches were all below the LOD (respective LOD as for the formulation in defatted rice bran).

Three batches of each formulation were analysed for bulk density, and results showed an average of 452 kg/m^3 (range 447–460 kg/m^3)[22] for the formulation in defatted rice bran and average of 619 kg/m^3 (range 619–663 kg/m^3) for the formulation in maltodextrin.[23] The dusting potential by the Stauber–Heubach method showed an average of 793 mg/m^3 (range 680–970 mg/m^3)[24] and 2,605 mg/m^3 (range 1,300–4,005 mg/m^3)[23] for three batches of the formulations in defatted rice bran and in maltodextrin, respectively.

The particle size distribution (by laser diffraction) of the same three batches of the formulation in defatted rice bran showed that approximately 4.9% of the particles have diameters < 100 μm (range 4.37–5.48%) and 1% of the particles have diameters < 50 μm (range 0.83–1.23%). No particles with diameter < 10 μm were found.[25] The particle size distribution for the formulation in maltodextrin showed that approximately 47% of the particles have diameters < 100 μm (range 40.4–56.7%), 32% of the particles with diameter < 50 μm (range 27.7–39.1%), 6.7% of the particles with diameter < 10 μm (range 6.1–7.8%) and 0.9% particles with diameter < 1 μm (range 0.8–1.1%).[23]

3.1.3. Stability and homogeneity

The shelf-life of the additive (1×10^8 CFU/g) was determined by monitoring three batches at 25°C/60% relative humidity (% RH) stored in paper bags for 14 months and then in closed plastic containers for further 24 months. No reduction in total bacilli count (< 0.5 log) was observed at the end of the experimental periods.[26]

[14] Technical dossier/Section II/Annex II.1.3.
[15] Technical dossier/Supplementary Information July 2021/Annex 1.1 and Annex 2.4.
[16] Technical dossier/Supplementary Information July 2021/Annex 8_1 and Technical dossier/Section II/Annex II_4_1f.
[17] Technical dossier/Supplementary Information July 2021/Annex 3.1.
[18] Technical dossier/Section II/Annex II.1.4.
[19] Technical dossier/Supplementary Information July 2021/Annex 4.1.: LOQs: Lead and mercury 0.05 mg/kg; Arsenic 0.1 mg/kg; aflatoxin B1 and G1 0.5μg/kg; aflatoxin B2 0.2 μg/kg; zearalenone 20 μg/kg; ochratoxin A 0.5 μg/kg, T2 toxin 2.5 μg/kg; HT-2 toxin 5 μg/kg; deoxynivalenol, fumonisins B1 and B2 100 μg/kg.
[20] Technical dossier/Section II/Annex II.1.4 and Technical dossier/Supplementary Information July 2021/Annex 4.1.
[21] Technical dossier/Section II/Annex II_4_1f, Supplementary Information July 2021/Annex 8_1, Supplementary Information November 2021_reply.
[22] Technical dossier/Section II/Annex II.1.5b.
[23] Technical dossier/Supplementary Information July 2021/Annex 8_1.
[24] Technical dossier/Section II/Annex II.1.5c.
[25] Technical dossier/Section II/Annex II.1.5a.
[26] Technical dossier/Supplementary Information July 2021/Annex 10_1.

One batch of the additive was mixed into three batches of a standard poultry vitamin/mineral premixture and samples were stored in closed plastic containers for 6 months at 25°C. No reduction in total bacilli count (< 0.5 log) was observed.[27]

The additive was incorporated into three samples of a standard maize and soy-based mash feed for poultry via a premixture to reach a concentration of the additive of 2×10^8 CFU/kg complete feed and tested for stability during pelleting at different temperatures (65°C, 75°C and 85°C). Samples of the mash and pelleted feeds were stored in closed plastic containers for 3 months at 25°C. No reduction in total bacilli count (< 0.5 log) was observed in either mash or pelleted feed after 3 months storage.[28]

The stability of the additive in the formulation in maltodextrin in water for drinking was studied in one batch when supplemented at 2.5×10^7 CFU/L. Samples were stored at room temperature (20–25°C)[29] for up to 48 h. Negligible losses (< 0.5 log) were observed.[30]

The capacity for homogeneous distribution of the additive in mash and pelleted feed was studied by adding one batch of the additive via a premixture to a standard maize and soy-based mash feed for poultry and then pelleting at 65°C and testing 10 subsamples. The coefficient of variation was 1.5% and 1.3% in mash and pelleted feed, respectively.[31]

The capacity for homogeneous distribution of the additive (one batch) in the formulation in maltodextrin in water for drinking was studied by analysing 10 subsamples. The intended concentration was 5.8×10^7 CFU/L, and the suspension was analysed after 2 h with and without agitation. The coefficient of variation was 6.9% and 6.8% with and without agitation, respectively.[30]

3.1.4. Conditions of use

BA-KING® is intended to be used in feed for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and all avian species for fattening, or rearing to slaughter or point of lay including non-food producing species at the minimum concentration of 5.0×10^7 CFU/kg complete feedingstuffs (defatted rice bran formulation) and 2.5×10^7 CFU/L in water (formulation in maltodextrin).

BA-KING® is intended for simultaneous use with diclazuril, decoquinate, halofuginone, monensin, salinomycin, narasin, robenidine and maduramicin.[32]

3.2. Safety

3.2.1. Safety for target animals, consumers and environment

The bacterial species *B. velezensis* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strain to be conclusively established and evidence provided that it does not harbour acquired antimicrobial resistance genes, that it lacks toxigenic potential and that it does not have the capacity to produce aminoglycosides. In the view of the FEEDAP Panel, the identity of the active agent was established and the compliance with the other qualifications confirmed. Therefore, the FEEDAP Panel concludes that *B. velezensis* NITE BP-01844 does not raise safety concerns for the target species, consumers of products derived from animals fed the additive and the environment. Since no concerns are expected from the other components of the additive, BA-KING® is also considered safe for the target species, consumers of products derived from animals fed the additive, and the environment.

3.2.2. Safety for user

3.2.2.1. Effects in the respiratory system

The dusting potential measured (up to 4,005 mg/m^3) suggests that exposure by inhalation is possible. Owing to the proteinaceous nature of the active agent, BA-KING® in any formulation should be considered a respiratory sensitiser.

[27] Technical dossier/Section II/Annex_II.4.1.d.
[28] Technical dossier/Section II/Annex_II.4.1.e.
[29] Technical dossier/Supplementary Information November 2021/Annex 3.1.
[30] Technical dossier/Section II/Annex_II.4.1.f.
[31] Technical dossier/Section II/Annex_II.4.2.
[32] Technical dossier/Supplementary Information July 2021.

3.2.2.2. Effects on eyes and skin

The skin irritation potential of BA-KING® (defatted rice bran formulation) was investigated using an *in vitro* test performed under Good Laboratory Practice (GLP) according to OECD Test Guideline 439.[33] Based on the results of this study, BA-KING® is classified as non-irritant to the skin (UN GHS no category).

The eye irritation potential of the additive (defatted rice bran formulation) was determined using an *in vitro* test, performed under GLP according to OECD Test Guideline 492.[34] The experiment was performed three times, due to borderline eye irritation in the first experiment. The second experiment showed no irritant effects, but the results were also close to the threshold. The third experiment confirmed the irritant results of the first experiment. According to the results, although no definitive UN GHS category (Category 1: 'serious eye damage' or Category 2: 'eye irritation') can be assigned, BA-KING® is considered to be irritant to eyes.

Although the studies above were done with the defatted rice bran formulation, the FEEDAP Panel considered that the same conclusions would apply also to the maltodextrin formulation.

No test of skin sensitisation was provided; therefore, the FEEDAP Panel cannot draw conclusions on the skin sensitisation potential of the additive.

3.2.2.3. Conclusions on safety for the user

The additive should be considered a respiratory sensitiser. BA-KING® is non-irritant to the skin but should be considered irritant to eyes. No conclusions can be drawn on the potential of the additive to cause skin sensitisation.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

A total of five trials performed in two trial sites with chickens for fattening and a pooled study (resulting from the pooling of three trials) were submitted. However, two of the studies[35] were not further considered as husbandry conditions where the birds were kept do not reflect the conditions in which the birds should be raised in the EU (regarding the bedding used) and were not in line with Directive 2007/43/EC[36].

The remaining three studies[37] were performed in the same location, at the same time, with an identical study design and with very similar diets. The FEEDAP Panel considered that the studies were not independent, thus only the outcome of the pooled studies was considered. The study design considering the pooling of the three individual studies is given in Table 1 and the results in Table 2.

Table 1: Trial design and use levels of the efficacy trials performed in chickens for fattening

Total No of animals (animals × replicate) replicates × treatment	Breed sex (duration)	Composition feed (form)	Groups (CFU/kg feed)		
			Intended	Starter Analysed	Grower Analysed
2,400 (30) 26/27	Ross 308 Males (42 d)	Maize, soya (mash)	0 5.0×10^7 2.0×10^8	$< 10^6$ $4.6–5.3 \times 10^7$ $1.4–2.5 \times 10^8$	$< 10^6$ $4.6–6.9 \times 10^7$ $2.0–2.9 \times 10^8$

CFU: colony forming unit.

In all trials, 1-day-old male birds (Ross 308) were used and grouped in pens. In all three studies, the animals were fed either a non-supplemented diet (control) or a diet containing *B. velezensis* NITE BP-01844 at different use levels of 5.0×10^7 and 2.0×10^8 CFU/kg of feed (confirmed by analysis). All studies included the minimum recommended concentration of 5.0×10^7 CFU/kg of feed. The diets were administered on *ad libitum* basis in mash form for 42 days. The health and mortality were monitored daily throughout the study and the body weight and feed intake were recorded. Mortality

[33] Technical dossier/Section III/Annex III.3.1.2.
[34] Technical dossier/Supplementary Information July 2021/Annex 6_1.
[35] Technical dossier/Section IV/Annex IV.3.5. and Annex IV.3.6.
[36] Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production, OJ L 182 12.7.2007, p. 19.
[37] Technical dossier/Section IV/Annex IV.3.1., Annex IV.3.2. and Annex IV.3.3.

adjusted-feed to gain ratio was calculated. An analysis of variance was done with the data from each single study using the pen as the experimental unit. Group means were compared with Tukey test. Significance level was set at < 0.05.

Table 2: Effects of BA-KING® on the performance of chickens for fattening

Groups (CFU/kg feed)	Daily feed intake (g)	Final body weight (g)	Average daily weight gain (g)	Feed to gain ratio	Mortality and culling (%)[38]
0	111.8	2,623[a]	60.8[a]	1.83[a]	3.09[b]
5.0×10^7	111.5	2,673[ab]	62.5[b]	1.78[b]	1.48[a]
2.0×10^8	111.5	2,682[b]	62.6[b]	1.78[b]	1.36[a]

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

Supplementing the diets of chickens with BA-KING® at either 5.0×10^7 or 2.0×10^8 CFU/kg feed significantly improved the feed to gain ratio and the average daily weight gain of the birds and reduced the mortality, when compared with the control group. A positive effect on final weight was only seen in the animals receiving the additive at 2.0×10^8 CFU/kg feed.

In the absence of three studies with significant positive results, the FEEDAP Panel is not in the position to conclude on the efficacy of BA-KING® for chickens for fattening, and consequently for any avian species.

3.3.2. Compatibility with coccidiostats

An *in vitro* study was conducted to support the compatibility of *B. velezensis* NITE BP-01844 with diclazuril, decoquinate, halofuginone, monensin, salinomycin, narasin, robenidine and maduramicin.[39] The MIC values were assessed using the broth microdilution method and showed values for diclazuril (> 4.8 mg/L), decoquinate (> 160 mg/L) and halofuginone (> 12 mg/L) greater than four times their maximum authorised levels in feed (1.2, 40 and 3 mg/kg, respectively). Therefore, *B. velezensis* NITE BP-01844 is compatible with diclazuril, decoquinate and halofuginone. The MIC values for the remaining coccidiostats (monensin: < 31.25 mg/L, salinomycin and narasin: < 17.5 mg/L, robenidine < 9 mg/L, maduramicin: 3 mg/L) were below four times the corresponding maximum authorised concentrations.[40]

An *in vivo* compatibility testing of *B. velezensis* NITE BP-01844 in chickens was also performed. A total of 420 one-day-old birds (Cobb 500), mixed sexes, were distributed into seven treatments (six replicates of 10 birds per treatment).[41] The treatments resulted from the supplementation of a basal diet in mash (maize, soya and soybean oil) in which BA-KING® was added at 5.0×10^7 CFU/kg without a coccidiostat or treated with either monensin (125 mg/kg), salinomycin (70 mg/kg), robenidine (36 mg/kg), maduramicin (6 mg/kg) or narasin (70 mg/kg). The negative control had no BA-KING® nor coccidiostats added. The diets were provided in mash form for 35 days. On day 21, six birds per treatment (one animal per replicate) were sacrificed, and ileal content sampled. On day 35, all remaining birds were killed and ileal content of 12 birds per treatment group was sampled (two animals per replicate). Bacteriological enumeration of the *Bacillus* vegetative cells (before heat treatment) and *Bacillus* spores (after heat treatment for 10 min at 80°C) in the ileal content was performed. Confirmation of the correspondence of the *Bacillus* spp. isolated from ileal content with the strain under assessment was done by polymerase chain reaction (PCR) and RAPD-PCR. Differences in counts between coccidiostat-treated groups and the control (< 0.6 $\log_{10}$ CFU/g) were not statistically significant, indicating that *B. velezensis* NITE BP-01844 is compatible with monensin, salinomycin, narasin, robenidine and maduramicin.

3.3.3. Conclusions on efficacy

The FEEDAP Panel is not in the position to conclude on the efficacy BA-KING® (*B. velezensis* NITE BP-01844) in chickens for fattening, and consequently for any avian species due to insufficient

[38] Technical dossier/Supplementary Information July 2021/Annex 7.1 and Technical dossier/Supplementary Information November 2021/Annex 5.1.
[39] Technical dossier/Section II/ Annex II.4.4a.
[40] Technical dossier/Section II/Annex II.4.4a.
[41] Technical dossier/Section II/Annex II.4.4b.

evidence. The FEEDAP Panel concludes that the *B. velezensis* NITE BP-01844 is compatible with diclazuril, decoquinate, halofuginone, monensin, salinomycin, narasin, robenidine and maduramicin.

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[42] and Good Manufacturing Practice.

4. Conclusions

BA-KING® is considered safe for the target species, consumers of products derived from animals fed with the additive, and the environment.

The additive is not irritant to skin but is considered irritant to eyes. The additive should be considered a respiratory sensitiser. No conclusions can be drawn on its potential to be a skin sensitiser.

In the absence of sufficient evidence, the FEEDAP Panel cannot conclude on the efficacy BA-KING® (*B. velezensis* NITE BP-01844) for the target species. *B. velezensis* NITE BP-01844 is compatible with diclazuril, decoquinate, halofuginone, monensin, salinomycin, narasin, robenidine and maduramicin.

5. Documentation as provided to EFSA/Chronology

	Event
15/07/2020	Reception mandate from the European Commission
23/09/2020	Dossier received by EFSA. BA-KING® (*Bacillus velezensis* NITE BP-01844) as a feed additive for chickens reared for laying, turkeys reared for breeding, all avian species for fattening or rearing to slaughter or point of lay including non-food producing species. Submitted by TOA BIOPHARMA Co. Ltd.
19/10/2020	Application validated by EFSA – Start of the scientific assessment
18/12/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
21/12/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety, efficacy*
20/01/2021	Comments received from Member States
16/07/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
05/10/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, efficacy*
05/11/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
02/12/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: general*
13/12/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
26/01/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

CFU	colony forming unit
EURL	European Union Reference Laboratory
GLP	Good Laboratory Practice
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
QPS	qualified presumption of safety
RAPD	Random Amplified Polymorphic DNA
WGS	whole genome sequence

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for *Bacillus amyloliquefaciens* TOA5001 (NITE BP-01844)

In the current application authorisation is sought under Article 4(1) for *Bacillus amyloliquefaciens*[43] TOA5001 (NITE BP-01844) under the category / functional group 4(b) 'zootechnical additives' / 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. The authorisation is sought for the use of the feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening, turkeys reared for breeding and all minor avian species.

According to the Applicant, the feed additive contains as active substance viable spores of the non-genetically modified strain *Bacillus amyloliquefaciens* TOA5001. The feed additive is to be marketed as a preparation (BA-KING®) containing a minimum content of active substance of 1×10^8 Colony Forming Unit (CFU)/g. The feed additive is intended to be added directly in feedingstuffs or through premixtures at a minimum dose of 5×10^7 CFU/kg complete feedingstuffs and in water for drinking at a minimum dose of 2.5×10^7 CFU/L.

For the identification of *Bacillus amyloliquefaciens* TOA5001, the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised methodology for genetic identification of bacterial strains.

For the enumeration of *Bacillus amyloliquefaciens* TOA5001 in the feed additive, premixtures, feedingstuffs and water the EURL recommends for official control the ring-trial validated spread-plate method EN 15784.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005), as last amended by Regulation (EU) 2015/1761) is not considered necessary.

[43] Originally designated as *Bacillus amyloliquefaciens* ssp. *plantarum,* the correct designation is *Bacillus velezensis*.