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Safety and efficacy of *Bacillus subtilis* DSM 28343 as a feed additive for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

The additive is a preparation containing viable spores of a strain of *Bacillus subtilis* which has never been authorised in the EU. It is intended to be used in feeds for chickens for fattening at the recommended dose of 1×10^9 colony-forming unit (CFU)/kg complete feedingstuffs. The bacterial species *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. As the identity of the active agent has been established and the susceptibility to antibiotics and lack of toxigenic potential have been demonstrated, the use of *Bacillus subtilis* DSM 28343 can be presumed safe for the target species, consumers of products derived from animals fed the additive and the environment. *Bacillus subtilis* DSM 28343 is not an eye/skin irritant but should be considered as a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the skin sensitisation potential. *Bacillus subtilis* DSM 28343 at the proposed dose has the potential to be efficacious in improving growth of chickens for fattening. *B. subtilis* DSM 28343 is compatible with the coccidiostats lasalocid A sodium, diclazuril, monensin sodium, maduramicin ammonium, decoquinate, nicarbazin, robenidine hydrochloride and halofuginone hydrobromide.

Keywords: Zootechnical additive, *Bacillus subtilis*, safety, efficacy, QPS, chickens for fattening, coccidiostats

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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 Lactosan GmbH & Co. KG.[2] for authorisation of the product *Bacillus subtilis* DSM 28343 (*Bacillus subtilis* DSM 28343), when used as a feed additive for chickens for fattening (category: Zootechnical additives; functional group: gut flora stabilisers). According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded to the European Food Safety Authority (EFSA) 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 this application. The particulars and documents in support of the application were considered valid by EFSA as of 24 April 2015.

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 *Bacillus subtilis* DSM 28343 (*Bacillus subtilis* DSM 28343), when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

The additive *Bacillus subtilis* DSM 28343 is a preparation containing viable spores of a strain of *Bacillus subtilis*. This additive has never been authorised in the European Union (EU).

The species *Bacillus 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.

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[3] in support of the authorisation request for the use of *Bacillus subtilis* DSM 28343 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[4] and the applicable EFSA guidance documents.

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.

2.2. Methodologies

The approach followed by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) to assess the safety and the efficacy of *Bacillus subtilis* DSM 28343 is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Guidance on zootechnical additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014), Technical guidance –

[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] Lactosan GmbH & Co. KG, Industriestrasse West 5, 8605, Kapfenberg, AT.
[3] FEED dossier reference: FAD-2015-0006.
[4] 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.

Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA FEEDAP Panel, 2012c), and Technical guidance on the compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA FEEDAP Panel, 2008).

3. Assessment

The additive is a preparation containing viable spores of a strain of *B. subtilis*. It is intended to be authorised as a zootechnical additive (functional group: gut flora stabilisers) in diets for chickens for fattening.

3.1. Characterisation

3.1.1. Characterisation of active agent

The *Bacillus subtilis* strain was isolated from hay and has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the accession number DSM 28343.[5] The strain has not been genetically modified.

Taxonomical identification of the product strain as *B. subtilis* was achieved by the sequence analysis of *gyrA* and 16S rRNA genes.[6] Strain-specific identification is based on the use of pulsed-field gel electrophoresis and genetic stability analysis was demonstrated by comparing the random amplified polymorphic DNA patterns of the deposited culture (master cell bank) with those of the working cell bank and of the cultures deriving from three different production batches.[7]

Cytotoxicity tests were performed using Vero cells and the 10-fold methanol or ammonium sulfate concentrated supernatants.[8] As no cytotoxic effect on Vero cells was detected, the strain is considered to be non-toxigenic.

Bacillus subtilis DSM 28343 was tested for antibiotic susceptibility using twofold broth dilutions.[9] The battery of antibiotics tested was that recommended by EFSA (EFSA FEEDAP Panel, 2012c). All minimum inhibitory concentration (MIC) values were equal or fell below the corresponding cut-off values defined by the FEEDAP Panel, and thus, no further investigation is needed.

3.1.2. Characterisation of the additive

The additive is described as a solid, free-flowing granulated product with a minimum content of spores of *Bacillus subtilis* DSM 28343 of 1×10^{10} colony-forming unit (CFU)/g. The carrier materials include calcium carbonate (93–95%), maltodextrins (2%) and silicon dioxide (1%).

Data on 10 batches of the additive showed that the minimum specification was slightly exceeded in all cases (mean value 1.3×10^{10} CFU/g, range $1.2–1.5 \times 10^{10}$ CFU/g).[10]

The additive is routinely monitored for microbial contamination at various points in the manufacturing process and in the final product. Analyses of three batches of the additive demonstrated compliance with the limits for Enterobacteriaceae (< 1,000 CFU/g), yeasts and filamentous fungi (< 1,000 CFU/g) and *Salmonella* (absent in 25 g).[11] The applicant claims that as raw materials used in the fermentation process are food and feed grade, no contamination with mycotoxins, heavy metals or arsenic is expected and no monitoring is carried out. To support this claim, three batches of the additive were tested for aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, zearalenone, deoxynivalenol, lead, cadmium, mercury and arsenic.[12] None of the mycotoxins were detected.[13] Lead showed a range of 0.42–0.53 mg/kg, arsenic of 0.28–0.32 mg/kg and cadmium and mercury showed values below the limit of quantification.[14] None of these concentrations give rise to concerns.

[5] Technical dossier/Section II/Annex II.2-1.
[6] Technical dossier/Section II/ Annexes II.2-2 and 3.
[7] Technical dossier/Section II/Annex II.2-4.
[8] Technical dossier/Section II/Annex II.2-5.
[9] Technical dossier/Section II/Annex II.2-6.
[10] Technical dossier/Section II/Annex II.1-2.
[11] Technical dossier/Section II/Annex II.1-3.
[12] Technical dossier/Section II/Annexes II.1-4 and II.1-5.
[13] Limits of detection: aflatoxin B1 < 0.03 μg/kg, aflatoxin B2 < 0.03 μg/kg, aflatoxin G1 < 0.03 μg/kg, aflatoxin G2 < 0.03 μg/kg, zearalenone < 0.005 mg/kg and deoxyvalenol < 0.01 mg/kg.
[14] Limits of quantification: cadmium < 0.20 mg/kg and mercury < 0.050 mg/kg.

One batch of *Bacillus subtilis* DSM 28343 was examined for particle size distribution by a laser diffraction and dusting potential with a Heubach dustometer.[15] Results showed that 9% by volume of the additive consists of particles with diameters below 50 μm and the same percentage of particles with diameters below 10 μm and that the dusting potential of the additive is 0.4 g/m^3.

3.1.3. Production process

The active agent is produced by fermentation using an industrial culture medium containing yeast extract and salts.[16] After sporulation, the fermentation broth is centrifuged in order to concentrate the cell mass and the resulting slurry is mixed with maltodextrins and spray-dried. After drying, the spores are mixed in the appropriate proportion and blended with excipients (calcium carbonate and silicon dioxide).

3.1.4. Stability and homogeneity

For the purpose of testing shelf-life, three batches of the additive were stored at 20°C for 12 months and at 37°C for 6 months.[17] Bacterial counts demonstrated the stability of the product over this period.

Stability of three batches of *Bacillus subtilis* DSM 28343 was tested when mixed with a mineral premixture and a minerals/vitamins premixture, and with mash and pelleted feed for chickens for fattening, and stored at 20°C for 6 and 3 months, respectively.[18] No significant differences were observed in the bacterial counts over these periods.

The stability to pelleting of a single batch of the additive when mixed with chickens feed was tested in triplicate at different temperatures (60, 70 and 80°C).[19] The enumeration of bacilli spores showed a recovery close to 90% or higher after the thermal treatment.

The ability of the additive to be uniformly distributed was examined using a single batch of the additive mixed into a pelleted feed for chickens.[19] Analyses of bacterial counts of 10 subsamples showed a coefficient of variation of 11%.

3.1.5. Conditions of use

The product is intended for use in feed for chickens for fattening at a minimum dose of 1×10^9 CFU/kg complete feedingstuffs.

The applicant claims that the additive can be used in poultry feed in conjunction with coccidiostats.

3.2. Safety

The species *B. subtilis* is considered by EFSA to be suitable for the QPS approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2013). In the view of the FEEDAP Panel, the identity of the *Bacillus subtilis* DSM 28343 is established and the toxigenic potential and the antibiotic resistance qualifications have been met. Therefore it is presumed safe for the target species, consumer and the environment.

3.2.1. Safety for the user

A study of acute dermal irritation with *Bacillus subtilis* DSM 28343 was performed following a protocol that conformed to Organisation for Economic Co-operation and Development (OECD) Guideline 404.[20] No reactions were seen in any of the animals after 72 h; thus, it is concluded that the additive is not a skin irritant.

A study of acute eye irritation with *Bacillus subtilis* DSM 28343 was performed using three male New Zealand White rabbits and following a protocol that conformed to OECD Guideline 405.[21] The test item produced a slight conjunctival redness in two animals and a chemosis in one animal that were

[15] Technical dossier/Section II/Annexes II.1-6–II.1-8.
[16] Technical dossier/Section II/Annexes II.3-1 and II.3-2.
[17] Technical dossier/Section II/Annex II.4-1.
[18] Technical dossier/Section II/Annex II.4-3.
[19] Technical dossier/Section II/Annex II.4-4.
[20] Technical dossier/Section III/ Annex III.3.4.
[21] Technical dossier/Section III/ Annex III.3-5.

fully reversible within 1 day. A slight opacity was also observed in the cornea of one animal, totally reversible within 2 days. Overall results indicate that the additive is not an eye irritant.

Skin sensitisation potential was not studied. Therefore, in the absence of data, no conclusion can be drawn on the skin sensitisation potential.

A small fraction (9%) of the particles of the product has the potential to reach the respiratory surface of the lungs when inhaled. Given the proteinaceous nature of the active agent, the additive should be considered to be a potential respiratory sensitiser.

3.2.1.1. Conclusions on safety for the user

Bacillus subtilis DSM 28343 is not an eye/skin irritant but should be considered as a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the skin sensitisation potential.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

Five efficacy studies lasting 35 days were performed in two Member States to demonstrate the efficacy of *Bacillus subtilis* DSM 28343 in improving performance of chickens for fattening. In all studies, a control group was compared with a group receiving the same basal diet supplemented with the additive at the minimum recommended dose (1×10^9 CFU/kg complete feedingstuffs).

The first study involved 2,240 one-day-old chickens (Ross 308, 50% females and 50% males) allocated to two groups (control and treatment). The treatment group received the additive at the recommended level (dose confirmed by analysis).[22] Each treatment was replicated eight times with 140 birds per replicate (Table 1). Birds were fed *ad libitum* diets based on maize, wheat and soybean according to the production stage (starter, grower and finisher). Weight was recorded on days 1 (per pen), 14, 24 and 35 (individually). Feed intake per pen was determined on days 14, 24 and 35, and feed to gain ratio (per pen) was calculated. Mortality was monitored daily. Data were subjected to one-way analysis of variance (ANOVA). The pen was the experimental unit for all parameters.

Table 1: Summary of performance data of chickens for fattening receiving *Bacillus subtilis* DSM 28343

N°	Total animals (Replicates/ treatment × animals/ replicate)	*Bacillus subtilis* DSM 28343 (CFU/kg feed)	Feed intake (g/day)	Final weight (kg)	Weight gain (g/day)	Feed: gain	Mortality (%)
1	2,240 8 × 140	0 1.0×10^9	92.6 94.9	2.00 2.03	55.8 56.7	1.61 1.63	2.0 1.2
2	240 6 × 20	0 1.0×10^9	94.4 94.5	2.17^a 2.24^b	60.8^a 62.7^b	1.55^a 1.51^b	2.5 1.7
3	224 6 × 18/19	0 1.0×10^9	93.0 94.6	2.19 2.24	61.4 62.9	1.54 1.53	2.7 0.9
4	241 6 × 20/21	0 1.0×10^9	95.6 99.7	2.20^a 2.28^b	61.7^a 63.9^b	1.56 1.56	0 0
5	657 16 × 20/21	0 1.0×10^9	94.7^a 98.5^b	2.14^a 2.18^b	61.0^a 62.4^b	1.57 1.59	4.3 2.4

CFU: colony-forming unit.
a, b: Means within a trial with a different superscript letters are significantly different ($p \leq 0.05$).

Mortality was in average 1.6% and not treatment related. Final weight, weight gain and feed to gain ratio were not influenced by treatment (Table 1).

The second study involved 240 male one-day-old chickens (Cobb 500) distributed in two groups (control and treatment). The treated group received the additive at the minimum recommended level (confirmed by analysis).[23] Birds within each group were randomly allocated to six pens of 20 animals each (Table 1). The trial was divided in two feeding periods, starter (1–14 days) and grower (15–35 days). Birds were fed maize/wheat/soybean meal diets (in mash form) provided *ad libitum*

[22] Technical dossier/Section IV/Annexes IV.1–IV.4.
[23] Technical dossier/Section IV/Annexes IV.5 and IV.6.

during the whole experimental period. Performance of birds was assessed by recording live weight, feed intake and mortality per pen at the start and end of the trial. Data on final weight and weight gain were subjected to one-way ANOVA, whereas for feed to gain ratio a Student *t*-test was used. The pen was the experimental unit for all parameters, giving six replicates per treatment.

Mortality was low (average 2.1%) and not treatment related. At the end of the experimental period, birds receiving the additive at the minimum recommended dose showed a significantly better growth rate and feed to gain ratio than birds in the control group (Table 1).

The third[24] and fourth[25] studies were conducted in the same location and shared an identical experimental design. In both studies, male one-day-old chickens (Ross 308) were distributed in two groups (control and treatment). The treated group received the additive at the minimum recommended level (confirmed by analysis). In study 3, 224 birds (112 per treatment group) were randomly allocated to 12 pens (six per treatment group) of 18/19 chickens. In study 4, 241 birds (121 in the control and 120 in the treated group) were randomly allocated to 12 pens (six per treatment group) of 20/21 chickens (Table 1). Birds were fed soybean meal/maize/wheat-pelleted diets, provided *ad libitum* for the entire duration of the study. Individual body weight was determined on days 0, 14 and 35, whereas feed intake per pen was recorded on a weekly basis. Data on final weight and weight gain were subjected to one-way ANOVA, whereas for feed to gain ratio a Student *t*-test was used. The pen was the experimental unit for all parameters, giving six replicates per treatment.

No mortality occurred in study 4, while in study 3 it was low (average 1.8%). At the end of the experimental period, birds receiving the additive at the minimum recommended dose showed a greater weight and weight gain than birds in the control, but these differences were significant only in study 4 (Table 1).

The last study involved 657 one-day-old chickens (Ross 308, 50% females and 50% males) distributed in two groups (control and treatment). The treated group received the additive at the minimum recommended level (confirmed by analysis of feed).[26] Birds within each group were randomly allocated to 32 pens of 20/21 animals each (Table 1). The trial was divided in two feeding periods, starter (1–14 days) and grower (15–35 days). Birds were fed soya/maize/wheat/soybean oil diets (in mash form) provided *ad libitum* during the whole experimental period. Individual live weight was recorded at start, on day 14 and at the end, and feed intake per pen was recorded weekly. Data on final weight and weight gain were subjected to one-way ANOVA, whereas for feed to gain ratio a Student *t*-test was used. The pen was the experimental unit for all parameters.

Mortality rate was low in both groups (average 3.4%). Feed intake, final weight and weight gain were significantly increased in the treated group compared with the control.

3.3.2. Compatibility with coccidiostats

An *in vitro* study in compliance with the Technical guidance on the compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA FEEDAP Panel, 2008) was conducted with lasalocid A sodium, diclazuril, monensin sodium, maduramicin ammonium, decoquinate, narasin/nicarbazin, narasin, nicarbazin, robenidine hydrochloride, salinomycin sodium and halofuginone hydrobromide. All coccidiostats were used at the corresponding maximum authorised dose.

As the MIC values of the coccidiostats were four times higher than the corresponding maximum authorised dose, *B. subtilis* 28343 is considered to be compatible with lasalocid A sodium, diclazuril, monensin sodium, maduramicin ammonium, decoquinate, nicarbazin, robenidine hydrochloride, and halofuginone hydrobromide. The MICs of narasin/nicarbazin, narasin and salinomycin were smaller than four times the corresponding maximum authorised dose. Therefore, compatibility with these coccidiostats could not be established.

3.3.3. Conclusions on efficacy for the target species

In three out of the five studies provided, the supplementation of *Bacillus subtilis* DSM 28343 at the minimum recommended dose improved the growth of chickens for fattening.

[24] Technical dossier/Section IV/Annexes IV-7–IV-10.
[25] Technical dossier/Section IV/Annexes IV-11–IV-14.
[26] Technical dossier/Supplementary information February 2016.

Bacillus subtilis DSM 28343 is compatible with the coccidiostats lasalocid A sodium, diclazuril, monensin sodium, decoquinate, nicarbazin, robenidine hydrochloride, maduramicin ammonium and halofuginone hydrobromide.

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[27] and good manufacturing practice.

4. Conclusions

The bacterial species *B. subtilis* is considered by EFSA to be suitable for the QPS approach to safety assessment. As the identity of the active agent has been established and the susceptibility to antibiotics and lack of toxigenic potential have been demonstrated, the use of *Bacillus subtilis* DSM 28343 can be presumed safe for the target species, consumers of products derived from animals fed the additive and the environment.

Bacillus subtilis DSM 28343 is not an eye/skin irritant but should be considered as a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the skin sensitisation potential.

Bacillus subtilis DSM 28343 at the proposed dose of 1×10^9 CFU/kg complete feedingstuffs has the potential to improve performance of chickens for fattening.

B. subtilis DSM 28343 is compatible with the coccidiostats lasalocid A sodium, diclazuril, monensin sodium, maduramicin ammonium, decoquinate, nicarbazin, robenidine hydrochloride and halofuginone hydrobromide.

Documentation provided to EFSA

1) *Bacillus subtilis* DSM 28343. March 2015. Submitted by Lactosan GmbH & Co. KG.
2) *Bacillus subtilis* DSM 28343. March 2015. Supplementary information. February 2016. Submitted by Lactosan GmbH & Co. KG.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for *Bacillus subtilis* DSM 28343.
4) Comments from Member States.

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EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2013;11(11):3449, 108 pp. doi:10.2903/j.efsa.2013.3449

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2008. Technical guidance prepared by the Panel on Additives and Products or Substances Used in Animal Feed (FEEDAP) on the compatibility of zootechnical microbial additives with other additives showing antimicrobial activity. EFSA Journal 2008;6(3):658, 5 pp. doi:10.2903/j.efsa.2008.658

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 2011;9(5):2175, 15 pp. doi:10.2903/j.efsa.2011.2175

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

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. doi:10.2903/j.efsa.2012.2740

[27] 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.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. doi:10.2903/j.efsa.2012.2740

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. doi:10.2903/j.efsa.2014.3665

Abbreviations

CFU	colony-forming unit
EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
QPS	qualified presumption of safety

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 subtilis* DSM 28343[1]

In the current application authorisation is sought under Article 4(1) for *Bacillus subtilis DSM 28343* under the category/functional group 4(b) 'zootechnical additives'/'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the feed additive for chickens for fattening.

According to the Applicant, the *feed additive* contains as active substance the viable spores of non-genetically modified *Bacillus subtilis* DSM 28343. The feed additive is to be marketed as cream-coloured free-flowing granules, containing a minimum *Bacillus subtilis* DSM 28343 concentration of 1×10^{10} Colony Forming Units (CFU)/g. The feed additive is intended to be used directly in *feedingstuffs* or through *premixtures* at a minimum dose of 1×10^{9} CFU/kg complete *feedingstuffs*.

For the identification of *Bacillus subtilis* DSM 28343 the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for genetic identification. This standard methodology for microbial identification is currently being evaluated by the CEN Technical Committee 327 to become a European Standard.

For the enumeration of *Bacillus subtilis* DSM 28343 in *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated spread plate method EN 15784 which was already evaluated by EURL in the frame of previous *bacilli* dossiers. Based on the performance characteristics available the EURL recommends for official control the CEN method (EN 15784) for the enumeration of *Bacillus subtilis DSM 28343* in the feed additive, premixtures and feedingstuffs.

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) is not considered necessary.

[1] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2015-0006-bacillus_subtilis.pdf