SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 4 July 2017

doi: 10.2903/j.efsa.2017.4933

Safety and efficacy of Alterion NE® (*Bacillus subtilis* DSM 29784) as a feed additive for chickens for fattening and chickens reared for laying

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Abstract

The additive Alterion NE® is a preparation containing viable spores of a strain of *Bacillus subtilis*. The additive is intended for use in feed for chickens for fattening and chickens reared for laying at the proposed dose of 1×10^8 CFU/kg complete feedingstuffs. *B. subtilis* is considered by EFSA to be suitable for the qualified presumption of safety approach to establish safety. As the identity of the active agent was established and the lack of toxigenic potential and of resistance to antibiotics of human or veterinary clinical significance demonstrated, the additive is presumed safe for the target species, consumers and the environment. Alterion NE® is not a dermal irritant but is irritant to eyes and should be considered a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the dermal sensitisation of the additive. Alterion NE® at the recommended dose 1×10^8 CFU/kg feed has the potential to improve the zootechnical performance of chickens for fattening. This conclusion can be extended to chickens reared for laying when used at the same dose. *B. subtilis* DSM 29784 is compatible with monensin sodium, narasin/nicarbazin, salinomycin sodium, lasalocid sodium, diclazuril, narasin, maduramicin ammonium, robenidine hydrochloride and decoquinate at the highest authorised levels.

Keywords: Alterion NE®, *Bacillus subtilis* DSM 29784, zootechnical additive, chickens, safety, QPS, efficacy

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Question number: EFSA-Q-2016-00448

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Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Villa RE, Wallace RJ, Wester P, Brozzi R and Saarela M, 2017. Scientific opinion on the safety and efficacy of Alterion NE® (*Bacillus subtilis* DSM 29784) as a feed additive for chickens for fattening and chickens reared for laying. EFSA Journal 2017;15(7):4933, 12 pp. https://doi.org/10.2903/j.efsa.2017.4933

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

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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 ADISSEO France SAS[2] for authorisation of the product Alterion NE® (*Bacillus subtilis* DSM 29784), when used as a feed additive for chickens for fattening (category: zootechnical additives; functional group: gut flora stabilisers). During the assessment, the applicant requested a change in the species by adding chickens reared for laying.

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 5 October 2016.

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 Alterion NE® (*Bacillus subtilis* DSM 29784) when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Alterion NE® is a preparation containing viable spores of *Bacillus subtilis* DSM 29784. It has not been previously authorised as a feed additive in the EU.

The bacterial 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, 2017). This approach requires the identity of the strain to be conclusively established and evidence that the strain lacks of toxigenic potential and 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 Alterion NE® (*Bacillus subtilis* DSM 29784) 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.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Alterion NE® 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, 2012a) Technical guidance on 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

[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] ADISSEO France SAS, Immeuble Anthony Parc II, 10 place du général de Gaulle 92160, Antony, France.
[3] FEED dossier reference: FAD-2016-0040.
[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.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2016-0040_alterionne.pdf

on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition (EFSA FEEDAP Panel, 2014), Technical guidance on the 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 – Compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA, 2008).

3. Assessment

The additive Alterion NE® is a preparation of *B. subtilis* DSM 29784 intended for use in feed for chickens for fattening and chickens reared for laying (category: zootechnical additives; functional group: gut flora stabilisers) to improve the performance of chickens.

3.1. Characterisation

3.1.1. Characterisation of active agent

The *B. subtilis* strain was originally isolated from soil and has not been genetically modified.[6] The strain has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen with the accession number DSM 29784.[7]

Taxonomical identification is based on the sequencing of the full 16S rRNA gene.[8] A 99.9% sequence similarity was demonstrated with other *B. subtilis* 16S rRNA genes in the consulted databases, which confirms the identity of the strain as *B. subtilis.* This was confirmed by the sequencing of the partial *gyrB* gene (1,200 bp). Genetic stability of the strain was confirmed by means of pulse field gel electrophoresis of the genomic restriction fragments.[9] Using this method, the master culture was compared with several generations of growth. No differences in the resultant patterns were observed.

Cytotoxicity of the strain was assessed on Vero cells using the culture supernatants in accordance to the FEEDAP Panel guidance document (EFSA FEEDAP Panel, 2014).[10] No detectable lysis of a Vero cell culture was detected upon exposure to culture supernatant from *B. subtilis* DSM 29784. Consequently, the strain is not considered toxigenic.

B. subtilis DSM 29784 was tested for antibiotic susceptibility using broth microdilution.[11] The battery of antibiotics tested was that recommended by EFSA (EFSA FEEDAP Panel, 2012c). All minimum inhibitory concentration (MIC) values were below the corresponding cut-off values defined by the FEEDAP Panel. Therefore, the strain is considered susceptible to all relevant antibiotics.

3.1.2. Characterisation of the additive[12]

The manufacturing process of the additive is detailed in the dossier. The additive has a final minimum guaranteed concentration of 1×10^{10} CFU of *B. subtilis* DSM 29784 per gram of additive.

Batch-to-batch variation was measured in five batches of the additive and found to be compliant with specifications (mean value: 1.7×10^{10} CFU/g, range: $1.5–1.9 \times 10^{10}$ CFU/g).[13]

All batches are routinely tested for microbiological contaminants (i.e. Enterobacteriaceae, *Escherichia coli*, yeasts and filamentous fungi and *Salmonella*) and the Hazard Analysis and Control of Critical Points (HACCP) plan foresees monitoring for chemical impurities (i.e. heavy metals, arsenic, aflatoxin, dioxins and total polychlorinated biphenyls (PCBs)). Analysis of four batches of the additive revealed that the levels of heavy metals (Pb $\leq$ 1.16 mg/kg, Hg $<$ 0.01 mg/kg and Cd $\leq$ 0.16 mg/kg), arsenic ($\leq$ 0.53 mg/kg), aflatoxin B1 ($<$ 0.1 μg/kg), dioxins (sum of polychlorinated dibenzoparadioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) = 0.13 ng WHO-PCDD/F-TEQ per kg), total dioxins (sum of PCDDs and PCDFs and PCBs = 0.19 ng WHO-PCDD/F-PCB-TEQ per kg), Enterobacteriaceae ($<$ 10 CFU/g), *E. coli* ($<$ 10 CFU/g), yeasts and filamentous fungi ($<$ 20 CFU/g) and

[6] Technical dossier/Section II/Annex II.2.2.
[7] Technical dossier/Section II/Annex II.2.1.
[8] Technical dossier/Section II/Annex II.2.3 and Supplementary information February 2017/Annex II.1.1.
[9] Technical dossier/Section II/Annex II.2.4.
[10] Technical dossier/Section II/Annex II.2.5.
[11] Technical dossier/Section II/Annex II.2.6 and Supplementary information February 2017.
[12] This section has been amended following the confidentiality claims made by the applicant.
[13] Technical dossier/Section II/Annex II.1.3.

Salmonella (absent in 25 g), do not give raise to concerns.[14,15] An analysis of three further batches confirmed the absence of *B. cereus* contamination (< 100 CFU/g).[16]

The product is a granular free-flowing powder. Four batches were examined for particle size distribution by laser diffraction and dusting potential with a Stauber–Heubach dustometer.[17] Results showed that 1.1% by volume of the additive consists of particles with diameters below 50 μm and that there are no particles with a diameter below 10 μm. The mean value for dusting potential was 0.06 g/m^3.

3.1.3. Stability and homogeneity

Shelf-life of three batches of Alterion NE® stored in its original packaging was examined at three conditions (25°C/60% relative humidity (RH), 30°C/65% RH and 40°C/75% RH).[18] Bacilli counts remained constant (losses < 0.5 log) at 40°C up to 6 months and at 25°C and 30°C up to 10 months, the last time points measured.

Stability of three batches of Alterion NE® was examined when incorporated (at 0.2%) in a minerals/vitamins premixture (containing choline chloride) and stored for 8 months at two conditions (25°C and 30°C) in sealed high-density polyethylene pouches.[19] Bacilli counts of two batches showed no variation overtime while the third batch showed a gradual loss of viability (0.5 log units at 8 months).

The stability of one batch of Alterion NE® to pelleting conditions (82, 88 and 93°C for 45 s) was tested when mixed at the proposed inclusion level with chickens' feed.[20] The enumeration of bacilli showed a recovery higher than 100% at all pelleting conditions. In a different study, the bacilli counts of three batches after a pelleting at 70°C showed a recovery close to 90% after the thermal treatment.

In the same study, stability of the additive (three batches) was tested when incorporated in mash and pelleted feed for chickens at the proposed dose and stored for 4 months at 25°C/RH 60% and 30°C/RH 65%.[21] No differences were seen in bacilli counts at the end of the storage period.

To test the capacity of Alterion NE® to be homogeneously incorporated in three batches of premixtures and mash and pelleted feed (one batch each), 10 subsamples were collected from the materials prepared in the tests described above.[22] Bacilli counts showed a coefficient of variation of 5–7% for the premixtures and pelleted feed and of 21% for the mash feed.

3.1.4. Conditions of use

The additive is intended for use in feed for chickens for fattening and chickens reared for laying at the minimum recommended dose of 1×10^8 CFU/kg feedingstuffs.

The additive is intended for use in the presence of the permitted coccidiostats: lasalocid sodium, robenidine hydrochloride, maduramicin ammonium, decoquinate, salinomycin sodium, monensin sodium, narasin, diclazuril and narasin/nicarbazin.

3.2. Safety

3.2.1. Safety for the target species, consumers and environment

In the view of the FEEDAP Panel, the identity of the active agent is established as *B. subtilis* and the toxigenic potential and the antibiotic resistance qualifications have been met. Therefore, based on the QPS approach to safety assessment, *B. subtilis* DSM 29784 is presumed safe for the target species, consumer of products from animals fed with the additive and the environment. No concerns are expected from other excipients present in the product, so Alterion NE® is also considered safe for target animals, consumers and the environment.

[14] Limits of quantification: Pb = 0.02 mg/kg, Hg = 0.01 mg/kg, Cd = 0.01 mg/kg, arsenic = 0.1 mg/kg, aflatoxin B1 = 0.0001 mg/kg, dioxins = 0.13 ng WHO-PCDD/F-TEQ per kg, total PCBs 0.19 ng WHO-PCDD/F-TEQ per kg, Enterobacteriaceae = 10 CFU/g, *E. coli* (< LOQ), yeasts and filamentous fungi = 20 CFU/g.
[15] Technical dossier/Section II/Annex II.1.5.
[16] Technical dossier/Supplementary information February 2017/Annex II.3.1.
[17] Technical dossier/Section II/Annexes II.1.10-11.
[18] Technical dossier/Section II/Annex II.4.1.
[19] Technical dossier/Section II/Annex II.4.2.
[20] Technical dossier/Section II/Annex II.4.4.
[21] Technical dossier/Section II/Annex II.4.3.
[22] Technical dossier/Section II/Annexes II.4.2-3.

3.2.2. Safety for the user

A small fraction of the particles (1.1%) has a diameter below 50 μm. Although the dusting potential is low, there is a potential exposure of the upper respiratory tract of people handling the product. Given the proteinaceous nature of the active agent, the additive should be considered to be a potential respiratory sensitiser.

The dermal irritation/corrosion potential of Alterion NE® was tested in an *in vitro* test (reconstructed human epidermis test according to the OECD guideline 439).[23] The mean viability of the skin membranes was 87% compared to the negative control group. Based on these results, Alterion NE® is non-irritant to skin.

The eye irritation/corrosion potential of Alterion NE® was tested with the *in vitro* isolated chicken eye test according to the OECD guideline 438.[24] Alterion NE® caused corneal effects consisting of slight corneal swelling or slight to moderate opacity and very slight or slight fluorescein retention.[22] Based on the results obtained in the present study, Alterion NE® is irritating to eyes.

In the absence of data, no conclusion can be drawn on the dermal sensitisation properties of the additive.

3.2.2.1. Conclusions on safety for the user

Alterion NE® is not a dermal irritant but is irritant to eyes and should be considered a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the dermal sensitisation of the additive.

3.3. Efficacy

3.3.1. Efficacy for chickens for fattening

Four floor pen studies were performed in two Member States and in two extra European countries (respecting European farming conditions) to demonstrate the efficacy of Alterion NE® in chickens for fattening.

The design of the studies is presented in Table 1 and the results in Table 2. In all cases, 1-day-old birds (male in studies in studies 1,[25] 2[26] and 4,[27] and the same number of males and females in study 3[28]) were allocated in randomised complete blocks to two treatment groups: a control group receiving a basal diet and a treatment group receiving the same basal diet supplemented with the additive at the recommended dose of 1×10^8 CFU/kg complete feedingstuffs (concentration was confirmed by analysis of feed). Each dietary treatment was replicated as shown in Table 1. The diets were offered to the animals *ad libitum*. Health status was monitored throughout the experimental periods. Feed intake and body weight of the animals were measured and feed to gain ratio was calculated. Data were analysed in a randomised complete block design with the birds being blocked by initial body weight at day 0. Performance data were subjected to an analysis of variance (ANOVA). The model included the diet and block as main factor in studies 1, 2 and 4 and the diet, gender and block as main factors, and interaction diet × gender in study 3. The experimental unit was the pen for all parameters. Data on mortality were analysed with Chi-square test.

[23] Technical dossier/Section III/Annex III.2.
[24] Technical dossier/Section III/Annex III.3.
[25] Technical dossier/Section IV/Annex 3.1 and Supplementary information/Annex 6.1 and 6.2.
[26] Technical dossier/Section IV/Annex 3.2 and Supplementary information/Annex 6.3 and 6.4.
[27] Technical dossier/Section IV/Annex 3.3 and Supplementary information/Annex 6.5 and 6.6.
[28] Technical dossier/Section IV/Annex 3.4 and Supplementary information/Annex 6.7 and 6.8.

Table 1: Details on the study design for the studies performed in chickens for fattening

Study no	Duration of the study (days)	Breed (sex)	Total animals replicates/ treatment × animals/ replicate	Alterion NE® (CFU/kg feed)	Basal diets (main ingredients) form
1	35	Ross PM3 ♂	390 13 × 15	0 1×10^8	Starter and grower (maize/soybean meal) pelleted
2	42	Cobb 500 ♂	1,040 13 × 40	0 1×10^8	Starter and grower/finisher (wheat/maize/soybean meal) pelleted
3	35	Cobb 500 ♀,♂	960 40[(a)] × 12	0 1×10^8	Starter, grower and finisher (maize/soybean meal) pelleted
4	42	Cobb 500 ♂	1,600 20 × 40	0 1×10^8	Starter, grower and finisher (maize/soybean meal) pelleted

CFU: colony forming unit.
(a): 20 pens of females and 20 pens of males.

Table 2: Summary of the overall performance results of the trials made with chickens for fattening

Study no	Alterion NE® (CFU/kg feed)	Feed intake (kg)	Final weight (kg)	Weight gain (kg/bird)	Feed:gain	Mortality (%)
1	0	3.66	2.29^b	2.25^b	1.63^a	4.1
	1×10^8	3.76	2.43^a	2.39^a	1.58^b	2.6
2	0	4.83^a	2.78	2.74	1.77^a	5.2
	1×10^8	4.75^b	2.81	2.77	1.71^b	4.2
3	0	2.98^a	1.94	1.90	1.56^a	0.4
	1×10^8	2.87^b	1.96	1.92	1.49^b	0.6
4	0	3.57	1.95^b	1.91^b	1.87^a	2.4
	1×10^8	3.57	2.06^a	2.02^a	1.77^b	3.6

a,b: Means in a column within a given trial with different superscript letters are significantly different $p < 0.05$.

Supplementation of Alterion NE® increased the final weight and weight gain of birds in two studies. Feed to gain ratio of birds receiving Alterion NE® was significantly improved compared to control birds in all four trials.

3.3.2. Efficacy for chickens reared for laying

The efficacy of Alterion NE® for chickens for fattening has been established. Since the mechanism of action of the additive can be reasonably assumed to be same, the above conclusions reached in chickens for fattening can be extended to chickens reared for laying.

3.3.2.1. Conclusions on efficacy for the target species

Alterion NE® at the recommended dose 1×10^8 CFU/kg feed has the potential to improve the zootechnical performance of chickens for fattening. This conclusion can be extended to chickens reared for laying when used at the same dose.

3.3.3. Compatibility with coccidiostats

An *in vivo* study was conducted to establish compatibility of *B. subtilis* DSM 29784 with lasalocid sodium, robenidine hydrochloride, maduramicin ammonium, decoquinate, salinomycin sodium, monensin sodium, narasin, diclazuril and narasin/nicarbazin.[29] The study involved 900 one-day-old male chickens (Ross 308) randomly allocated to 10 treatments, each replicated nine times and with ten birds per replicate. The treatments were:

[29] Technical dossier/Section II/Annex II.4.5.

- T1: Control (Alterion NE® at 1×10^8 CFU/kg feed – no coccidiostat)
- T2: Alterion NE® at 1×10^8 CFU/kg feed + monensin sodium at 125 mg/kg feed
- T3: Alterion NE® at 1×10^8 CFU/kg feed + narasin/nicarbazin at 50/50 mg/kg feed
- T4: Alterion NE® at 1×10^8 CFU/kg feed + salinomycin sodium at 70 mg/kg feed
- T5: Alterion NE® at 1×10^8 CFU/kg feed + lasalocid A sodium at 125 mg/kg feed
- T6: Alterion NE® at 1×10^8 CFU/kg feed + diclazuril at 1 mg/kg feed
- T7: Alterion NE® at 1×10^8 CFU/kg feed + narasin at 70 mg/kg feed
- T8: Alterion NE® at 1×10^8 CFU/kg feed + maduramicin ammonium at 6 mg/kg feed
- T9: Alterion NE® at 1×10^8 CFU/kg feed + robenidine hydrochloride at 36 mg/kg feed
- T10: Alterion NE® at 1×10^8 CFU/kg feed + decoquinate at 40 mg/kg feed

Concentration in feed was confirmed by analysis. The trial lasted 42 days during which birds were followed for weight gain, feed intake and mortality. Average daily gain (ADG) and feed to gain ratio were calculated. At the end of the experiment, 100 animals (10/treatment group) were killed and ileal and caecal digesta samples collected and analysed for *Bacillus* counts, with and without heat treatment in order to differentiate between the vegetative cells and spores.

The data on final weight, body weight gain, feed intake and feed to gain ratio were analysed with ANOVA. The model included the diet as main factor. The *B. subtilis* counts on ileal and caecal samples were analysed using the Dunnett T-test for paired comparison with the control. The pen was the experimental unit for all parameters. The results are given in Table 3.

Table 3: Ileal and caecal *Bacillus subtilis* counts from chickens for fattening treated with the additive and different coccidiostats

Treatment	Log CFU *Bacillus subtilis*/g ileum content		Log CFU *Bacillus subtilis*/g caecum content	
	– Heat treatment	+ Heat treatment	– Heat treatment	+ Heat treatment
Control	4.8	4.8	4.6	4.7
Monensin sodium	4.7	4.7	4.4	4.5
Narasin/nicarbazin	4.7	4.7	4.5	4.6
Salinomycin sodium	4.6	4.6	4.4	4.4
Lasalocid A sodium	4.8	4.8	4.6	4.5
Diclazuril	4.7	4.6	4.4	4.4
Narasin	4.8	4.8	4.4	4.4
Maduramicin ammonium	4.7	4.7	4.6	4.7
Robenidine hydrochloride	4.6	4.6	4.6	4.6
Decoquinate	4.6	4.7	4.4	4.5

CFU: colony forming unit.

No significant differences were observed on any of the performance parameters measured. Mortality was low (3.7% on average) and not influenced by treatment.

There were no significant differences between the control and any of the treated groups in *Bacillus* counts.

3.3.3.1. Conclusions on compatibility with coccidiostats

B. subtilis DSM 29784 is compatible with monensin sodium, narasin/nicarbazin, salinomycin sodium, lasalocid sodium, diclazuril, narasin, maduramicin ammonium, robenidine hydrochloride and decoquinate at the highest authorised levels.

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[30] and Good Manufacturing Practice.

[30] 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.

4. Conclusions

The active agent fulfils the requirements of the QPS approach to the assessment of safety and no concerns are expected from other components of the additive. Consequently, Alterion NE® can be presumed safe for the target species, consumers of products derived from animals fed the additive and the environment.

Alterion NE® is not a dermal irritant but is irritant to eyes and should be considered a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the dermal sensitisation of the additive.

Alterion NE® at the recommended dose 1×10^8 CFU/kg feed has the potential to improve the zootechnical performance of chickens for fattening. This conclusion can be extended to chickens reared for laying when used at the same dose.

B. subtilis DSM 29784 is compatible with the coccidiostats monensin sodium, narasin/nicarbazin, salinomycin sodium, lasalocid sodium, diclazuril, narasin, maduramicin ammonium, robenidine hydrochloride and decoquinate at the highest authorised levels.

Documentation provided to EFSA

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2) Alterion NE® for chickens for fattening. Supplementary information. February 2017. Submitted by ADISSEO France S.A.S.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Alterion NE®.
4) Comments from Member States.

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Abbreviations

ADG	average daily gain
ANOVA	analysis of variance
CFU	colony-forming unit

DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EURL	European Union Reference Laboratory
HACCP	Hazard Analysis and Control of Critical Points
LOQ	limit of quantification
MIC	minimum inhibitory concentration
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzoparadioxins
PCDFs	polychlorinated dibenzofurans
QPS	qualified presumption of safety
RH	relative humidity

Appendix A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Alterion NE®

In the current application authorisation is sought under Article 4(1) for *Bacillus subtilis* DSM 29784 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 viable spores of non-genetically modified *Bacillus subtilis* DSM 29784. The *feed additive* is to be marketed as light brown to brown granular free flowing powder, containing a minimum *Bacillus subtilis* DSM 29784 content of 1×10^{10} colony forming units per gram of *feed additive* (CFU/g). The *feed additive* is intended to be used directly in feedingstuffs or through premixtures at a minimum dose of 1×10^{8} CFU/kg of complete *feedingstuffs*.

For the identification of *Bacillus subtilis* DSM 29784 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 29784 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 for the enumeration of *Bacillus subtilis* DSM 29784 in the three matrices.

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.