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Safety and efficacy of Sorbiflore® ADVANCE (*Lactobacillus rhamnosus* CNCM I-3698 and *Lactobacillus farciminis* CNCM I-3699) as a feed additive for chickens for fattening

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Abstract

Following a request from the European Commission, the EFSA 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 Sorbiflore® ADVANCE when used as a zootechnical feed additive for chickens for fattening. Sorbiflore® ADVANCE is an additive resulting from the fermentation of milk-based broth with *Lactobacillus rhamnosus* CNCM I-3698 and *Lactobacillus farciminis* CNCM I-3699. Sorbiflore® ADVANCE is intended for use in feed for chickens for fattening at the minimum concentration of 5×10^7 Viable Forming Units (VFU)/kg and the maximum concentration of 2×10^8 VFU/kg complete feed. The data submitted did not allow a full characterisation of the additive, and therefore, uncertainty remains on the nature of the product in terms of viability, on the ratio between the active agents and on the stability and homogeneity of the additive. The active agents fulfil the requirements of the Qualified Presumption of Safety (QPS) approach to the assessment of safety and no concerns are expected from other components of the additive. Consequently, Sorbiflore® ADVANCE is presumed to be safe for the target animals, consumers of products from animals receiving the additive and the environment. The additive should be considered a respiratory sensitiser. In the absence of data, no conclusions can be drawn on the irritancy of Sorbiflore® ADVANCE to skin and eyes and on its dermal sensitisation potential. Sorbiflore® ADVANCE at 2×10^8 VFU/kg feed has the potential to be efficacious in chickens for fattening.

Keywords: zootechnical additives, Sorbiflore ADVANCE, *Lactobacillus rhamnosus* CNCM I-3698, *Lactobacillus farciminis* CNCM I-3699, safety, efficacy, chickens for fattening

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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 STI Biotechnologie[2] for authorisation of the product Sorbiflore® ADVANCE (*Lactobacillus rhamnosus* CNCM I-3698 and *Lactobacillus farciminis* CNCM I-3699), when used as a feed additive for chickens for fattening (category: Zootechnical additives; functional group: Other zootechnical additives).

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 22 May 2018.

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 Sorbiflore® ADVANCE (*L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699), when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

Sorbiflore® ADVANCE is an additive containing *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699. EFSA has issued one opinion on the safety and efficacy of Sorbiflore® when used with piglets (EFSA, 2008) and one on the safety and efficacy of Sorbiflore® ADVANCE when used with the same target species (EFSA FEEDAP Panel, 2020a). EFSA issued an opinion on the safety and efficacy of a microbial product containing *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699 when used as a silage additive for all animal species (EFSA FEEDAP Panel, 2020b).

The product Sorbiflore® consisting of *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699 with a minimum concentration of 1×10^8 FU/g was authorised for use in feed for piglets at the minimum concentration of 5×10^8 FU/kg complete feed and the maximum concentration of 9×10^8 FU/kg complete feed.[3,4,5] This authorisation expired on 8 January 2019.

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[6] in support of the authorisation request for the use of Sorbiflore® ADVANCE 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.[7] The method of analysis proposed by the applicant was considered fit for purpose according to the proposed conditions of use of the additive, in particular as regards the

[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] STI Biotechnologie, Zone Artisanale du Coglais, 35460 St. Etienne en Coglès, France.
[3] Commission Regulation (EC) No 1290/2008 of 18 December 2008 concerning the authorisation of a preparation of *Lactobacillus rhamnosus* (CNCM-I-3698) and *Lactobacillus farciminis* (CNCM-I-3699) (Sorbiflore) as a feed additive. OJ L 340, 19.12.2008, p. 20.
[4] Commission Regulation (EC) No 899/2009 of 25 September 2009 amending Regulation (EC) No 1290/2008 as regards the name of the holder of the authorisation of a preparation of *Lactobacillus rhamnosus* (CNCM-I-3698) and *Lactobacillus farciminis* (CNCM-I-3699) (Sorbiflore). OJ L 256, 29.9.2009, p. 11.
[5] Commission Regulation (EC) No 1334/2013 of 13 December 2013 amending Regulation (EC) No 1290/2008 as regards the name of the holder of the authorisation and as regards the recommended dose of a preparation of *Lactobacillus rhamnosus* (CNCM-I-3698) and *Lactobacillus farciminis* (CNCM-I-3699) (Sorbiflore). OJ L 256, 29.9.2009, p. 11.
[6] FEED dossier reference: FAD-2017-0066.
[7] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2017-0066-lact_rham_farc.pdf

verification of the concentration of the active agents (total lactobacilli (LAB) counts) in the additive, feedingstuffs and premixture. This method of analysis is not suitable to identify at strain level the non-cultivable cells of *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699 and therefore, to properly characterise the additive (compliance with 1:1 ratio of *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699, see Section 3.1.1).

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Sorbiflore® ADVANCE is in line with the principles laid down in Regulation (EC) No 429/2008[8] 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 characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

3. Assessment

Sorbiflore® ADVANCE containing *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699 is intended to be used as a zootechnical additive (other zootechnical additives) in feed for chickens for fattening, in order to improve their performance.

3.1. Characterisation

3.1.1. Characterisation of the additive

Sorbiflore® ADVANCE is an additive resulting from the fermentation of a milk-based broth with *L. rhamnosus* CNCM I-3698 and *L. farciminis* CNCM I-3699. The manufacturing process foresees a two-stage process in which cultures are first grown separately in skimmed milk-based medium and then used to inoculate the production broth, in which the skimmed milk content is increased and molasses are added. Subsequently, the co-culture is mixed with the carrier materials to reach the final composition of 10% biomass, 60.4% extruded corn meal, 11.34% soybean meal, 10% micronised wheat hulls, 7.2% algae meal, 1% silicon oxide[9] and 0.06% of an antifungal preservative based on calcium propionate, sodium diacetate and calcium formate with a mineral carrier.

The applicant describes the product as containing viable but not cultivable cells of the two strains in a 1:1 ratio, with a minimum total lactobacilli (LAB) number of 5×10^8 Viable Forming Units (VFU)/g additive.[10] In the dossier reference is made also to other intermediate formulations (e.g. SOCO containing 3×10^8 VFU/g additive) used in some efficacy studies.

To characterise the additive and confirm the inclusion level in the feed used in the efficacy studies, the applicant has developed a method [redacted].[11]

Compliance with the specifications, in terms of total lactobacilli counts, was confirmed by analysis of 10 batches (mean value of 5.7×10^8 VFU/g, range= 5.2–6.5 $\times 10^8$ VFU/g, coefficient of variation CV = 8.5%), using the PMA-qPCR method.[12] Individual counts of the two strains were tested in the same 10 batches to support the declared qualitative and quantitative (1:1 ratio) composition.[13]

The Panel notes that following the EURL report on the methodology and the data provided by the applicant, the unambiguous discrimination between the two lactobacilli strains is not possible, which does not allow to conclude on the ratio between the two strains in the product. Therefore, the method does not allow a full characterisation of the additive, and uncertainty remains on the nature of the product in terms of viability, on the ratio between the active agents and on its stability and homogeneity.

[8] 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.
[9] Feed additive currently under re-evaluation.
[10] Technical dossier/Supplementary information April 19/FAD-2017-0066.
[11] Technical dossier/Section II/Annexes II-8[redacted]
[12] Technical dossier/Section II/Annex II-8.
[13] Technical dossier/Section II/Annex II-18.

Three batches of the additive were analysed for microbial contaminants.[14] Results confirm compliance with limit levels (coliforms < 500 CFU/g, *Salmonella* absence in 25 g, *Listeria monocytogenes* absence in 25 g, yeasts and filamentous fungi $< 1.5 \times 10^4$ CFU/g). Chemical contaminants were analysed in one batch of the additive (lead < 1.0 mg/kg, arsenic < 1,0 mg/kg, cadmium < 0.25 mg/kg, mercury < 0.01 mg/kg, aflatoxins B1, B2, G1 and G2 < 0.01 mg/kg, deoxynivalenol: 1.11 mg/kg, ochratoxin A < 0.01 mg/kg).[15,16]

Five batches of the additive were analysed for dusting potential with the Stauber-Heubach dustmeter. Results showed values of 36–83 g/m^3 (average: 58 g/m^3).[17]

3.1.2. Characterisation of the active agents

Both strains of the additive were isolated from the rumen of healthy goats and deposited in the National Micro-organism Collection of Pasteur Institute (CNCM, Paris) with the accession numbers CNCM I-3698 and CNCM I-3699.[18]

The full genome sequence of both strains was obtained and used for characterisation purposes. [redacted] Although the analysis was not conducted in full compliance with the relevant FEEDAP Guidance, [redacted] the data are deemed sufficient to identify the strains as *L. rhamnosus* and *L. farciminis*.

The susceptibility of both strains to the antibiotics recommended by the relevant FEEDAP Guidance (EFSA FEEDAP Panel, 2018) was tested by broth microdilution.[20] All the minimum inhibitory concentration (MIC) values for *L. rhamnosus* CNCM I-3698 were lower than the EFSA cut-off values; consequently, the strain is considered to be susceptible to these relevant antibiotics. The only exception was the MIC for chloramphenicol which was exceeded by two dilutions (MIC: 16 mg/L vs. cut-off value: 4 mg/L).

The applicant provided two sets of data on WGS interrogation for the presence of antimicrobial resistance (AMR) genes. [redacted]

Regarding *L. farciminis* CNCM I-3699, the MIC values of for ampicillin, gentamicin and streptomycin were below or equal to the corresponding EFSA cut-off values; consequently, the strain is considered to be susceptible to these relevant antibiotics. The MIC values for the remaining antibiotics were exceeded by one or more dilutions (i.e. vancomycin by several dilutions (MIC: > 128 mg/L vs. cut-off value: 2 mg/L), erythromycin by more than three dilutions (> 8 mg/L vs. 1 mg/L), kanamycin and tetracycline by two dilutions (64 vs. 16 μg/mL and 16 mg/L vs. 4 mg/L) and clindamycin and chloramphenicol and by one dilution (8 mg/L vs. 4 mg/L). Exceedance of the cut-off value by one dilution is considered to be within the normal range of variation and thus, not a matter of concern. In order to analyse the elevated MICs observed for erythromycin, kanamycin and tetracycline, the genome interrogation was performed as described above. [redacted] these resistances are not related to the presence of acquired resistance genes and are considered of no concern.

[14] Technical dossier/Section II/Annex II-9.
[15] Technical dossier/Supplementary information April 19/Annex 3.
[16] Limit of detection and/or limit of quantification not provided.
[17] Technical dossier/Supplementary information April 19/Annex 5.
[18] Technical dossier/Section II/Annex II-11.
[redacted]
[20] Technical dossier/Section II.
[redacted]

3.1.3. Stability and homogeneity

The shelf-life of Sorbiflore ADVANCE® during storage was studied using three batches stored for 12 months at 25°C/60% relative humidity (RH) and at 40°C/75 RH% (packaging during storage was not described).[23] Losses in total LAB counts (measured using the qPCR PMA-coupled method) were negligible (< 0.5 log) in the first case after 12 months whilst it reached 0.8 log after 6 months in the second case.

Three batches of Sorbiflore ADVANCE® were individually mixed into a commercial vitamin–mineral premixture (composition or target species not provided) at a concentration of 1.3×10^{11} VFU/kg and samples were stored for 6 months at the same conditions described above.[24] Results showed that numbers of LAB in the vitamin–mineral premix after 6 months were within $\pm$ 0.5 $\log_{10}$ of the time zero count when stored at 25°C, but were 0.6 $\log_{10}$ when stored at the higher temperature.

Stability in complete feed was investigated using a batch of Sorbiflore ADVANCE® incorporated at two inclusion levels (5×10^7 VFU/kg and 2×10^8 VFU/kg) into a typical mash feed (barley, soybean meal, wheat and maize) and into a pelleted feed of the same composition (pelleting conditions 90°C for 30 s).[25] Samples of the mash and pelleted feed were stored at two ambient conditions (25°C/60 RH% and at 40°C/75 RH%) and LAB counts were made at month intervals up to 3 months. Essentially no reduction in counts was seen in either the mash feed or the pelleted feed.

A separate study was conducted to investigate the effect of pelleting on viability.[26] Sorbiflore® ADVANCE was incorporated at two inclusion levels (1.3×10^8 VFU/kg and 5×10^8 VFU/kg) in the mash feed subjected to pelleting at 90°C. Differences in counts obtained by comparison of the LAB counts before and after pelleting were small and less than 0.5 $\log_{10}$ at both concentrations.

The capacity of the additive to homogeneously mix with feed and premixtures for piglets was established in the previous opinion (EFSA, 2008). Given the commonality of feed ingredients in diets for chickens for fattening, the FEEDAP Panel is of the opinion that the existing data are sufficient to establish the capacity to homogeneously mix of the additive in premixtures and feeds for this category.

3.2. Conditions of use

The applicant proposes using Sorbiflore® ADVANCE in feed for chickens for fattening at the minimum concentration of 5×10^7 VFU/kg and the maximum concentration of 2×10^8 VFU/kg complete feed.

3.3. Safety

3.3.1. Safety for the target species, consumers and environment

The bacterial species *Lactobacillus rhamnosus* and *Lactobacillus farciminis* are considered by EFSA to be potentially suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2020). This approach requires the identity of the strains to be conclusively established and evidence that the strains do not harbour acquired antimicrobial genes to clinically relevant antibiotics. In the view of the FEEDAP Panel, the antibiotic resistance qualification has been met and the identity of the strains established as *L. farciminis and L. rhamnosus*. Therefore, both active agents are considered by EFSA to be suitable for the QPS approach to safety assessment and, consequently, are presumed safe for the target species, consumers of products from animals fed the additive and the environment. Since other components of the additive are not expected to raise safety concerns,[27] Sorbiflore® ADVANCE is also presumed safe for the target animals, consumers and the environment.

3.3.2. Safety for the user

Despite the request, no information was provided on the potential inhalation toxicity of the additive or on its skin/eye irritation and skin sensitisation potential. The dustiness of the preparations tested indicated a potential for users to be exposed via inhalation to be likely. Given the proteinaceous nature

[23] Technical dossier/Section II/Annexes II-19 and 20.
[24] Technical dossier/Section II/Annexes II-21 and 22.
[25] Technical dossier/Section II/Annexes II-23 and 24.
[26] Technical dossier/Section II/Annex II-25.
[27] Silicon oxide currently under re-evaluation.

of the active agents, the additive should be considered a respiratory sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy of Sorbiflore® ADVANCE to skin and eyes and on its dermal sensitisation potential.

3.4. Efficacy for chickens for fattening

Four studies were conducted in two Member States to assess the effects of the supplementation of Sorbiflore® ADVANCE to chickens for fattening. However, one study[28] could not be further considered due to poor reporting.

Study 1[29] was conducted with a form of the additive called Sorbiflore® containing a minimum concentration of 1×10^8 VFU/g additive, study 2[30] with two forms, Sorbiflore® and a form 3 times more concentrated called SOCO. The third study[31] was conducted with Sorbiflore® ADVANCE. In all cases, the nominal concentration of active agents in feed was confirmed by analysis using the PMA-qPCR method.[32] The design of the studies is presented in Table 1 and the results in Table 2. The first two studies were intended to identify an effective dose of the additive and therefore included several inclusion levels. The third study included the additive at the minimum and maximum recommended concentration. The three trials involved 1-day-old Ross 308 chickens randomly distributed in three or more experimental groups, one receiving the basal diets (unsupplemented) and the others receiving the basal diet supplemented with the additive at different concentrations. Birds were fed ad libitum and had free access to water. In studies 1 and 2 observations included body weight and feed intake per pen at start, 21 and 42 days. In the third study, weight of birds was monitored at start and at 10, 20 and 43 days and pen feed intake on a daily basis. From these, weight gain and feed to gain ratio were calculated. Morbidity and mortality of birds were monitored during the whole experimental period in all cases. Performance data were analysed using analysis of variance as a completely randomised design with the pen as experimental unit. Significance was established at $p \leq 0.05$.

Table 1: Details on the study design for the trials performed in chickens for fattenin

Trial no	Sorbiflore® (VFU/kg feed)		Total number of animals No of replicates per treatment × No of birds per replicate (sex)	Duration (days)	Basal diets (main ingredients) form
	Intended inclusion level	Analysed level			
1	0 5×10^7 1×10^8 2×10^8	0 5.1×10^7 1×10^8 2×10^8	1,056 12 × 22 (50% ♂ and 50% ♀)	42	Soybean/wheat/barley/maize (mash)
2(1)	0 1×10^8 2×10^8 5×10^7 1×10^8 2×10^8	0 1×10^8 2×10^8 5×10^7 1×10^8 2×10^8	1,056 8 × 22 (50% ♂ and 50% ♀)	42	Wheat/barley/maize/soybean meal (mash)
3	0 5×10^7 2×10^8	0 5.3/6.2/ 5.8×10^7 2/3.1/ 2.2×10^8	720 12 × 20 (♂)	43	Wheat/maize/sorghum/ extruded soybean (mash and pelleted)

(1): This study included the two forms of the additive, the first and second group received Sorbiflore® and the last three groups received SOCO.

[28] Technical dossier/Section IV/Annexes IV.7-10.
[29] Technical dossier/Section IV/Annexes IV 1-3 and Supplementary information April 19/Annexes 6 and 7.
[30] Technical dossier/Section IV/Annexes IV.4–6 and Supplementary information April 19/Annexes 7 and 8.
[31] Technical dossier/Supplementary information April 19/Annexes 9-13.
[32] Technical dossier/Supplementary information April 19/Annexes 6-8.

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

Trial no	Sorbiflore® (VFU/kg feed)	Daily feed intake (g)	Final weight (kg)	Average daily gain (g)	Feed to gain (g/g)	Mortality (%)
1	0	101.3^{a}	2.29	53.5	1.89^{a}	6.8
	5×10^7	100.6^{ab}	2.37	55.4	1.81^{b}	6.4
	1×10^8	101.3^{a}	2.35	55.0	1.84^{ab}	2.7
	2×10^8	97.1^{b}	2.28	53.4	1.82^{b}	6.4
2(1)	0	106.6^{a}	2.40	56.2	1.90^{a}	6.2^{a}
	1×10^8	105.0^{ab}	2.43	56.9	1.84^{a}	7.9^{a}
	2×10^8	101.5^{c}	2.44	57.3	1.78^{b}	5.7^{a}
	5×10^7	104.0^{abc}	2.38	55.8	1.87^{a}	5.7^{a}
	1×10^8	101.9^{c}	2.36	55.2	1.85^{a}	4.0^{a}
	2×10^8	102.9^{bc}	2.49	58.3	1.77^{b}	15.9^{b}
3	0	120.6^{a}	3.05	69.4	1.77^{a}	0
	5×10^7	117.4^{b}	3.12	71.3	1.65^{b}	0
	2×10^8	117.8^{b}	3.16	71.0	1.67^{b}	0

Different superscript letters are significantly different ($p \leq 0.05$).
(1): This study included the two forms of the additive, the first and second group received Sorbiflore® and the last three groups received SOCO.

Mortality of birds was normal, except in two of the treatment groups of study 2 receiving the additive at 1×10^8 VFU/kg feed and at the maximum recommended level, where it was abnormally high (7.9 and 15.9, respectively). In the first case, none of the performance parameters were significantly improved, except feed intake. In second case, most of the animals died during the first 3 weeks of the experiment (mortality in the period 0–21 day was 14.2%). Although this mortality was probably due to poor management of the animals and was not related to the test item, the results of this group were not further considered in the assessment.

Supplementation of the additive improved the feed to gain ratio when administered at the minimum inclusion level (5×10^7 VFU/kg feed) in two studies (1 and 3) and at the maximum inclusion level (2×10^8 VFU/kg feed) in the three studies considered. Therefore, it can be concluded that Sorbiflore® ADVANCE at 2×10^8 VFU/kg feed has the potential to be efficacious in chickens for fattening.

4. Conclusions

The data produced do not allow a full characterisation of the additive, and therefore, uncertainty remains on the nature of the product in terms of viability, on the ratio between the active agents and on the stability of the additive.

The active agents fulfil the requirements of the QPS approach to the assessment of safety and no concerns are expected from other components of the additive. Consequently, Sorbiflore® ADVANCE can be presumed to be safe for the target animals, consumers of products derived from animals fed with the additive and the environment.

The dusting potential of the additive is high. The additive should be considered a respiratory sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy of Sorbiflore® ADVANCE to skin and eyes or on its dermal sensitisation potential.

Sorbiflore® ADVANCE at 2×10^8 VFU/kg feed has the potential to be efficacious in chickens for fattening.

5. Documentation as provided to EFSA/Chronology

Date	Event
09/11/2017	Dossier received by EFSA. Dossier name. Submitted by STI Biotechnologie
23/11/2017	Reception mandate from the European Commission
22/05/2018	Application validated by EFSA – Start of the scientific assessment

Date	Event
20/06/2018	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*
25/07/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: method of analysis*
19/10/2018	Comments received from Member States
14/05/2019	Reception of supplementary information from the applicant
14/06/2019	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*
12/09/2019	Reception of supplementary information from the applicant
15/10/2019	Reception of supplementary information from the applicant - Scientific assessment re-started
18/10/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
19/03/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

References

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADI	average daily intake
AMR	antimicrobial resistance
BW	body weight
CFU	colony-forming unit
CV	coefficient of variation
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	Additives and products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification
MIC	minimum inhibitory concentration
qPCR	quantitative Polymerase Chain Reaction
RH	relative humidity
VFU	viable forming unit

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Sorbiflore® ADVANCE

In the current application, authorisation is sought under Article 4(1) for *Lactobacillus rhamnosus* (CNCM I-3698) and *Lactobacillus farciminis* (CNCM I-3699) under the category/functional group 1(k) 'technological additives'/'silage additives' (*Sorbensyl*)[33] and under the category/functional group 4(b) 'zootechnical additives'/'gut flora stabilisers' (*Sorbiflore® Advance*),[34] according to Anne× I of Regulation (EC) No 1831/2003. Authorisation is sought for the use of the *feed additive* in *silage* for all animal species (*Sorbensyl*) and in *feedingstuffs* for chickens for fattening (*Sorbiflore® Advance*).

According to the Applicant, both feed additives i.e. *Sorbensyl and Sorbiflore® Advance* are of identical composition and contain as active substances viable but non-cultivable cells of the non-genetically modified strains *L. rhamnosus* (CNCM I-3698) and *L farciminis* (CNCM I-3699). These products are to be marketed as a powder containing equal amounts of both *Lactobacillus* spp. strains (CNCM I-3698 and CNCM I-3699) with a minimum total content of 5×10^8, so-called Forming Unit (FU)/g.

Sorbensyl is intended to be added to silage at a minimum dose of 2.5×10^7 or of 8×10^7 FU/kg of fresh silage, depending on the raw material ensiled. Sorbiflore® Advance is intended to be used directly in feedingstuffs or through premixtures at a minimum dose of 5×10^7 FU/kg of complete feedingstuffs.

For the quantification of *L. rhamnosus* (CNCM I-3698) and *L. farciminis* (CNCM I-3699) in the feed additives, premixtures and feedingstuffs, the Applicant submitted a single laboratory validated and further verified (for feedingstuffs) method based on real-time quantitative Polymerase Chain Reaction (qPCR). Based on the available performance characteristics, the EURL recommends this method for official control for the quantification of the overall *Lactobacillus* spp. (CNCM I-3698 and CNCM I-3699) in the feed additives, premixtures and feedingstuffs.

The Applicant did not provide any experimental method or data for the quantification of the *Lactobacillus* spp. (CNCM I-3698 and CNCM I-3699) in silage. Since the unambiguous quantification of *L. rhamnosus* (CNCM I-3698) and *L. farciminis* (CNCM I-3699) added to silage is not achievable by analysis, the EURL cannot evaluate nor recommend any method for official control to quantify the active substances in silage.

The Applicant did not provide any method suitable for the identification at strain level of non-cultivable cells of *L. rhamnosus* (CNCM I-3698) and *L. farciminis* (CNCM I-3699) present in the different feed matrices; thus, the EURL cannot evaluate nor recommend any method for official control to identify at strain level the target active substances in the feed additive, silage, 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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

[33] FAD 2017-0064.
[34] FAD 2017-0066.