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Safety and efficacy of Bonvital® (*Enterococcus faecium* DSM 7134) as a feed additive for laying hens

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Abstract

Following a request from the European Commission, EFSA Panel on Additives and Products or Substances used in Animal Feed was asked to deliver a scientific opinion on the safety and efficacy of Bonvital® for laying hens. Bonvital® is an additive containing viable cells of *Enterococcus faecium* DSM 7134 marketed in two forms, a granular and a powder form, both with a guaranteed minimum concentration of *E. faecium* DSM 7134 of 1.0×10^{10} colony forming units (CFU)/g additive. Bonvital® in either form is intended for use in feed for laying hens at the minimum concentration of 1.0×10^{9} CFU/kg complete feed and at the maximum concentration of 1.0×10^{10} CFU/kg feedingstuffs. Bonvital powder® is also proposed for use in water for drinking at the minimum concentration of 5.0×10^{8} CFU/L. The use of Bonvital® in animal nutrition is considered safe for the target animals. The results of a tolerance trial in which hens were fed the additive at 10-fold the maximum recommended dose support this conclusion. Delivery of comparable doses of the additive via water for drinking is considered as safe for laying hens. Bonvital® at the proposed conditions of use is safe for consumers of products derived from animals fed the additive and for the environment. Bonvital® is not a dermal or ocular irritant but a potential dermal and respiratory sensitiser. Bonvital® has the potential to be efficacious in improving the hen's performance when supplemented at 1.0×10^{9} CFU/kg feed or 5.0×10^{8} CFU/L water for drinking.

Keywords: zootechnical additive, gut flora stabiliser, Bonvital®, *Enterococcus faecium* DSM 7134, hens, safety, efficacy

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Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

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 Bonvital® (*Enterococcus faecium* DSM 7134), when used as a feed additive for laying hens (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 2 July 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 Bonvital® (*Enterococcus faecium* DSM 7134), when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

EFSA issued several opinions on the product when used with chickens for fattening (EFSA, 2004, 2009a; EFSA FEEDAP Panel, 2010), piglets and pigs for fattening (EFSA, 2007a; EFSA FEEDAP Panel, 2019a), sows (EFSA, 2007b; EFSA FEEDAP Panel, 2014, 2019b), dogs (EFSA, 2009b) and chickens reared for laying and minor avian species (EFSA FEEDAP Panel, 2013a). EFSA issued an opinion on the safety and efficacy of a microbial product containing *Enterococcus faecium* DSM 7134 and *Lactobacillus rhamnosus* when used in feed for calves for rearing (EFSA FEEDAP Panel, 2013b).

Bonvital® is currently authorised as a zootechnical additive (functional group: gut flora stabiliser) for use in piglets, pigs for fattening,[3] sows,[4] chickens for fattening,[5] chickens reared for laying and minor poultry species other than those used for laying (4b1706).[6] The active agent *E. faecium* DSM 7134 is also authorised in combination with *Lactobacillus rhamnosus* DSM 7133 under a different trade name for calves for rearing.[7]

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Lactosan GmbH & Co.KG, Industriestr. West 5, A-8605 Kapfenberg, Austria.
[3] Commission Implementing Regulation (EU) 2020/159 of 5 February 2020 concerning the renewal of the authorisation of *Enterococcus faecium* DSM 7134 as a feed additive for weaned piglets and pigs for fattening and repealing Regulation (EC) No 538/2007 (holder of authorisation Lactosan Starterkulturen GmbH & Co). OJ L 34, 6.2.2020, p. 22.
[4] Commission Implementing Regulation (EU) No 1083/2014 of 15 October 2014 concerning the authorisation of a preparation of *Enterococcus faecium* DSM 7134 (Bonvital) as a feed additive for sows. OJ L 298, 16.10.2014, p. 5 and Commission Implementing Regulation (EU) 2019/1315 of 2 August 2019 concerning the authorisation of a preparation of *Enterococcus faecium* DSM 7134 as a feed additive (in water for drinking) for sows (holder of authorisation Lactosan GmbH & Co), OJ L, 205, 5.8.2019, p. 7.
[5] Commission Regulation (EU) No 998/2010 of 5 November 2010 concerning the authorisation of *Enterococcus faecium* DSM 7134 as a feed additive for chickens for fattening (holder of the authorisation Lactosan GmbH & Co KG. OJ L 290, 6.11.2010, p. 22.
[6] Commission Implementing Regulation (EU) No 775/2013 of 12 August 2013 concerning the authorisation of a preparation of *Enterococcus faecium* DSM 7134 as a feed additive for chickens reared for laying and minor poultry species other than those used for laying (holder of authorisation Lactosan GmbH & Co KG). OJ L 217, 13.8.2013, p. 32.
[7] Commission Implementing Regulation (EU) No 1101/2013 of 6 November 2013 concerning the authorisation of a preparation of *Enterococcus faecium* DSM 7134 and *Lactobacillus rhamnosus* DSM 7133 as a feed additive for calves for rearing and amending Regulation (EC) No 1288/2004 (holder of authorisation Lactosan GmbH & Co KG). OJ L 296, 7.11.2013, p. 1.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[8] in support of the authorisation request for the use of Bonvital® (*Enterococcus faecium* DSM 7134) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Bonvital® (*Enterococcus faecium* DSM 7134) 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, 2012) and Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011).

3. Assessment

Bonvital® is an additive consisting of viable cells of *Enterococcus faecium* DSM 7134 intended for use as a zootechnical additive (gut flora stabilisers) in feed and water for drinking for laying hens.

3.1. Characterisation

3.1.1. Characterisation of the additive

Bonvital® is an additive containing viable cells of *E. faecium* DSM 7134 marketed in two forms:

- Bonvital powder®: composed of cell concentrate (3%), carrier (sweet whey powder, 96%), lactose (0.5%), sodium citrate (0.1%), sodium glutamate (0.1%), sodium ascorbate (0.05%), sodium lactate (0.2%) and mannitol (0.05%) with a guaranteed minimum concentration of *E. faecium* DSM 7134 of 1×10^{10} CFU/g additive.[10]
- Bonvital granules®: microencapsulated formula composed of cells concentrate (3%), saccharose (70%), maltodextrin (20%), sodium citrate (1.0%), sodium glutamate (1.0%), sodium ascorbate (0.5%), sodium lactate (2.5%), mannitol (1.5%) and starch (0.5%), with a guaranteed minimum concentration of *E. faecium* DSM 7134 of 1×10^{10} CFU/g additive.[11]

Since the additive under application has the same formulation and method of manufacture as that considered in previous opinions (EFSA, 2004, 2007a,b; EFSA FEEDAP Panel, 2013a, 2014, 2019b), the data pertaining to impurities, physical properties, shelf life, stability and capacity to homogeneously disperse in feed and water still apply.

3.1.2. Conditions of use

Bonvital® in either form is intended for use in feed for laying hens at the minimum inclusion level of 1.0×10^9 and at the maximum inclusion level of 1.0×10^{10} CFU/kg feedingstuffs. Bonvital powder® is also proposed for use in water for drinking at the minimum concentration of 5.0×10^8 CFU/L.

3.2. Safety

In a previous opinion (EFSA FEEDAP Panel, 2013a), *E. faecium* DSM 7134 was unambiguously identified and found not to belong to the hospital-associated clade and not to express resistance to clinically relevant antibiotics. The metabolic end products of the species are those typical of lactic acid bacteria and do not raise concerns. Therefore, the use of *E. faecium* DSM 7134 in animal nutrition is

[8] FEED dossier reference: FAD-2018-0024.
[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2008-0007.pdf
[10] Technical dossier/Section II/Annex II.1-9.
[11] Technical dossier/Section II/Annex II.1-10.

not expected to raise concerns for the consumers of animal products. Since neither the active agent nor the other components of the additive give rise to concerns, the FEEDAP Panel considers the use of Bonvital® safe for the consumers.

In the same opinion, the Panel also concluded that since *E. faecium* is a natural component of gut microbiota, its use as Bonvital® in animal feeding is not expected to pose any risk for the environment. The Panel considers these conclusions to apply also in the current assessment.

In the same opinion, Bonvital® was found to be not a dermal and ocular irritant, but a potential dermal and respiratory sensitiser. The use of the additive in laying hens is considered unlikely to introduce hazards for users of the product not already considered as part of that assessment. Therefore, the conclusions reached in the previous assessment also apply to the current application.

No new information has been provided, except for a tolerance trial with laying hens described below. The FEEDAP Panel is not aware of any new information that would lead it to reconsider the conclusions drawn previously and considers that the extension of use to the new species for which the application is made would not have an impact on the safety aspects already evaluated.

The trial was made with 112 pullets (Lohmann Classic, 23-week-old) kept in 16 pens (of 7 animals each) and randomly assigned to three treatment groups after a 30-day acclimatisation phase.[12] The treatment groups were a control group receiving the basal diet (based on wheat, soybean meal and maize) with no additive, and two Bonvital® groups (form not specified), one receiving the additive at the minimum recommended level of 1×10^9 CFU/kg feed and the other at 100-fold the minimum recommended level or 10-fold the maximum recommended level (1×10^{11} CFU/kg feed). The intended inclusion level of the additive in the feed was confirmed by analysis. The control group included 6 pens and the two Bonvital® groups, 5 pens each. Feed and water were provided *ad libitum* for 56 days. Body weight was recorded at the start and at the end of the trial (day 56). Feed intake was measured on a weekly basis, eggs were collected and weighed daily, and egg mass was calculated on a daily basis. Feed to egg mass ratio was also calculated. Mortality and general health of hens were monitored throughout the study. An analysis of variance (ANOVA) or a non-parametric test (Kruskal–Wallis if not normally distributed) was carried out with the data. For performance data, the treatment was the main effect. For the life weight, the individual data were used but the pen was included as a random effect in the model. Means of the three groups were compared with least significance difference or by pairs with the Bonferroni–Holm test (or U test for not normally distributed data). The statistical significance level was set at $p < 0.05$.

During the trial 3, hens died (1 in the control and 2 in the 1× group). No significant differences were found in any of the measured parameters between groups (mean values for daily feed intake: 111 g/day, final body weight: 1.85 kg, laying rate 93.4%, egg weight: 59.7 g, egg mass per hen and per day: 55.7 g and feed to egg mass ratio (kg feed per kg egg mass): 1.99).

The level proposed for use in water for drinking would provide essentially the same exposure as that proposed in feedingstuffs. Consequently, the conclusions on safety of the additive when used in feedingstuffs also apply to use in water for drinking for laying hens.

Considering the nature of the active agent and the composition of the additive, the use of Bonvital® in animal nutrition is considered safe for the target animals. The results of a tolerance trial support this conclusion. Delivery of comparable doses of the additive via water for drinking is considered as safe for laying hens.

3.3. Efficacy

Four trials were conducted in two Member States and in a third country to investigate the effects of Bonvital® on the performance of laying hens. However, one was disregarded due to poor reporting.[13]

The three trials considered shared the same experimental design that consisted on the comparison of the performance of a control group of layers with that of another group receiving the additive at the minimum recommended inclusion level of 1.0×10^9 CFU/kg feed. The intended inclusion level of the additive in the feed was confirmed by analysis. Hens were fed *ad libitum* diets based on wheat, maize, soybean meal (trial 1[14]), wheat, soybean meal and maize (trial 2[15]) or wheat, soybean and

[12] Technical dossier/Section III/Annexes III.1-1 and III.1-2.
[13] Technical dossier/Section IV/Annex IV.8-11.
[14] Technical dossier/Section IV/Annex IV.1-4 and Supplementary information May 2020/Annex 3.
[15] Technical dossier/Section IV/Annex IV.5-7 and Supplementary information May 2020/Annex 3.

maize (trial 3[16]). The total numbers of layers used and number of replicates per treatment for each trial are shown in Table 1 and the breeds used were Bovans Brown (trial 1), ISA brown (trial 2) and Lohmann Brown-Classic (trial 3). The hens were randomly allocated to cages at 16 (trial 1) or 18 weeks of age (trials 2 and 3) and allowed a period of acclimatisation of 4 or 6 weeks during which they were fed a basal diet. The start of the experiments was at 20, 22 and 24 weeks of age, for trials 1, 2 and 3, respectively, and the duration was 24 weeks (168 days) in studies 1 and 2 and 26 weeks (182 days) in study 3.

Individual liveweight, egg production and mass, feed consumption and feed to egg mass ratio were measured or calculated. An ANOVA or a non-parametric test (Mann–Whitney if not normally distributed in studies 2 and 3) was carried out with the data. For performance data, the treatment was included in the model as a fixed effect. The statistical significance level was set at $p < 0.05$.

Table 1: Effects of Bonvital® on the feed intake, laying performance and mortality of laying hens

Trial	Total number of animals No of replicates per treatment × no of birds per replicate	Bonvital® CFU/kg feed	Daily feed intake (g)	Laying rate (%)	Daily egg mass (g/hen)	Feed to egg mass	Mortality and culling (%)
1	960 16 × 30[1]	0 1 × 10^9	113.5 114.1	90.3[b] 92.1[a]	52.6[b] 53.8[a]	2.16[a] 2.12[b]	1.1 2.7
2	112 8 × 7	0 1 × 10^9	101.9[b] 105.6[a]	93.6[b] 98.6[a]	58.6[b] 61.9[a]	1.74 1.71	1.8 3.8
3	91 7/6[2] × 7	0 1 × 10^9	112.1 110.6	92.3 96.7	58.7 61.5	1.91[a] 1.80[b]	2.0 4.8

CFU: colony forming unit.
[1]: 2 hens died in the control during acclimatisation.
[2]: 1 pen from the Bonvital® group was disregarded due to an abnormal mortality rate.
[a,b]: Mean values within a trial with a different superscript are significantly different at $p < 0.05$.

Mortality of hens remained within acceptable values and did not significantly differ between groups in any of the trials. The hens in the Bonvital® group showed a significantly greater laying rate and daily egg mass in two trials (1 and 2), compared to control. Feed to egg mass ratio was significantly better in the hens fed with Bonvital® compared to control in two trials (1 and 3) and in the remaining trial (2) the improvement was only numerical due to a greater feed intake observed in the Bonvital® group.

The level proposed for use in water for drinking (5.0×10^8 CFU/L) would provide essentially the same exposure as that proposed in complete feed. Consequently, the conclusions on efficacy of the additive when used in feedingstuffs also apply to use in water for drinking for laying hens.

3.3.1. Conclusions on efficacy for laying hens

Bonvital® has the potential to be efficacious in improving the hens' performance when supplemented at 1×10^9 CFU/kg feedingstuffs. Delivery of comparable doses of the additive via water for drinking is considered as efficacious for laying hens.

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[17] and Good Manufacturing Practice.

4. Conclusions

Bonvital® at the proposed conditions of use is safe for the laying hens, consumers of products derived from animals fed the additive and the environment.

Bonvital® is not a dermal or ocular irritant but a potential dermal and respiratory sensitiser.

[16] Technical dossier/Supplementary information January 2020/Annexes II, III and IV and Supplementary information May 2020/Annexes 1-3 and LH_01_19_without Pen 11.
[17] 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.

Bonvital® has the potential to be efficacious in improving the hen's performance when supplemented at 1×10^9 CFU/kg feed or 5.0×10^8 CFU/L water for drinking.

5. Documentation as provided to EFSA/Chronology

Date	Event
27/04/2018	Dossier received by EFSA. Bonvital for laying hens. Submitted by Lactosan GmbH & Co.KG
18/05/2018	Reception mandate from the European Commission
02/07/2018	Application validated by EFSA – Start of the scientific assessment
03/10/2018	Comments received from Member States
22/11/2018	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: efficacy*
24/01/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
30/09/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013b. Scientific Opinion on the safety and efficacy of Provita LE (*Enterococcus faecium* and *Lactobacillus rhamnosus*) as a feed additive for calves for rearing. EFSA Journal 2013;11(4):3175, 14 pp. https://doi.org/10.2903/j.efsa.2013.3175

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Abbreviations

ANOVA	analysis of variance
CFU	colony forming unit
EURL	European Union Reference Laboratory