European Food Safety Authority

The EFSA Journal (2009) 990, 1-12

SCIENTIFIC OPINION

Safety and efficacy of Bonvital (*Enterococcus faecium*) as feed additive for chickens for fattening[1]

Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed

(Question No EFSA-Q-2008-289)

Adopted on 3 March 2009

PANEL MEMBERS

Georges Bories, Paul Brantom, Joaquim Brufau de Barberà, Andrew Chesson, Pier Sandro Cocconcelli, Bogdan Debski, Noël Dierick, Jürgen Gropp, Ingrid Halle, Christer Hogstrand, Joop de Knecht, Lubomir Leng, Sven Lindgren, Anne-Katrine Lundebye Haldorsen, Alberto Mantovani, Miklós Mézes, Carlo Nebbia, Walter Rambeck, Guido Rychen, Atte von Wright and Pieter Wester

SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the safety and efficacy of the product Bonvital used as feed additive for chickens for fattening.

The microbial feed additive Bonvital is a preparation of *Enterococcus faecium*. This product is authorised for use in piglets, pigs for fattening, sows and chickens for fattening (until April 2009). The applicant is now seeking authorisation for use of this product as a zootechnical additive (functional group: gut flora stabilisers) in feed for chickens for fattening at a dose range of 0.5×10^9–2×10^9 CFU kg^{-1} of complete feedingstuff.

Data from four studies were provided, only two of which included the minimum recommended dose. Efficacy was demonstrated at a dose of around 0.5×10^9 CFU kg^{-1} feed in two studies. In a field trial with 34500 birds, the effect seen in the Bonvital group could not be used as evidence of efficacy due to the weaknesses of the experimental design. The FEEDAP Panel considers the data insufficient to finally conclude on the efficacy of Bonvital in chickens for fattening.

The compatibility of Bonvital has been demonstrated with the coccidiostats robenidine hydrochloride, maduramicin ammonium, diclazuril, decoquinate, halofuginone hydrobromide, monensin sodium and lasalocid A sodium.

[1] For citation purposes: Scientific Opinion of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety and efficacy of Bonvital (*Enterococcus faecium*) as feed additive for chickens for fattening. *The EFSA Journal* (2009) 990, 1-12

The FEEDAP Panel was unable to conclude on the safety of Bonvital for chickens for fattening in the absence of studies meeting the relevant requirements.

Key words: zootechnical additive, gut flora stabiliser, Bonvital, *Enterococcus faecium*, chickens for fattening, safety for the target animals, efficacy, compatibility with coccidiostats

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1831/2003[2] 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 the company Lactosan GmbH & Co KG [3] for authorisation of the product Bonvital (*Enterococcus faecium)* to be used as a feed additive for chickens for fattening (category: zootechnical additives; functional group: gut flora stabilisers) under the conditions mentioned in Table 1. 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). EFSA received directly from the applicant the technical dossier in support of this application.[4] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 18 July 2008.

The additive Bonvital is a preparation of *Enterococcus faecium* (DSM 7134). This product is authorised for use in piglets and pigs for fattening (4b1841),[5] for sows,[6] and for chickens for fattening (until 5 April 2009).[7] The strain *Enterococcus faecium* (DSM 7134) in combination with *Lactobacillus rhamnosus* (DSM 7133) is also authorised for calves and piglets (E1706).

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety for pigs for fattening and calves, the consumer, user and environment of a microbial product containing *Enterococcus faecium* (DSM 7134) and *Lactobacillus rhamnosus* (DSM 7133) (EC, 1997, updated 2003) and another opinion on the safety of *Enterococcus faecium* (DSM 7134) for piglets, pigs for fattening and sows (EC, 2003).

EFSA published an opinion on the safety of Bonvital (*Enterococcus faecium* DSM 7134) for chickens for fattening (EFSA, 2004), an opinion on the safety and efficacy for piglets and pigs for fattening (EFSA, 2006) and an opinion on the safety and efficacy for sows (EFSA, 2007).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the efficacy and the safety for the target animal(s), consumer and user and the environment of the product Bonvital, *Enterococcus faecium* DSM 7134, when used under the conditions described in Table 1.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on Micro-organisms for the preparation of this opinion.

[2] OJ L 268, 18.10.2003, p.29
[3] Lactosan GmbH & Co KG, Industriestrasse West 5, 8605 Kapfenberg, Austria
[4] Dossier reference: FAD-2008-0007
[5] OJ L 128, 16.5.2007, p.16
[6] OJ L 335, 20.12.2007, p.24
[7] OJ L 84, 1.4.2005, p.3

Table 1. **Register entry as proposed by the applicant**

Additive	*Enterococcus faecium* (DSM 7134)
Registration number/EC No/No (if appropriate)	22 and 4b/1841
Category of additive	Zootechnical
Functional group of additive	Gut flora stabiliser

Description			
Composition, description	**Chemical formula**	**Purity criteria (if appropriate)**	**Method of analysis (if appropriate)**
Preparation of *Enterococcus faecium* containing a minimum of: Powder: 1 x 10^{10} CFU/g additive Granules (micro-encapsulated): 1 x 10^{10} CFU/g additive		Impurities: Fungi < 100 cfu/g Clostridia < 10 cfu/g Enterobacteria < 10 cfu/g Salmonella none detectable in 25g	**Quantification of lactic acid bacteria content (Code of the method:** LAC-DO-EF1A)

Trade name (if appropriate)	Bonvital
Name of the holder of authorisation (if appropriate)	Lactosan Starterkulturen GmbH & Co Kg

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		mg or Units of activity or CFU kg^{-1} of complete feedingstuffs		
Chickens for fattening		0.5 x 10^9	2.0 x 10^9	Without time limit

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Can be used with the following coccidiostats: Deccox, Stenorol, Avatec 150G, Elancoban G200, Cycostat 66G, Cygro 1%, Clinacox 0.5%
Specific conditions or restrictions for handling (if appropriate)	The directions for use must indicate storage temperature, shelf life
Post market monitoring (if appropriate)	
Specific conditions for use in complementary feedingstuffs (if appropriate)	The directions for use must indicate pelleting stability both of the additive and the premixture

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**

ASSESSMENT

1. Introduction

The additive Bonvital is a preparation of *Enterococcus faecium* (DSM 7134), supplied as a powder or granulated product, containing a minimum of 1 x 10^{10} CFU per gram of product.

This product is authorised for use in piglets and pigs for fattening, and in sows. The strain *Enterococcus faecium* (DSM 7134) in combination with *Lactobacillus rhamnosus* (DSM 7133) is also authorised for calves and piglets (see Background).

The current dossier contains data supporting a request for authorisation under Regulation (EC) No 1831/2003 for use of Bonvital as a feed additive (zootechnical additive: gut flora stabiliser) for chickens for fattening.

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the safety for pigs for fattening and calves, the consumer, user and environment of a microbial product containing *Enterococcus faecium* (DSM 7134) and *Lactobacillus rhamnosus* (DSM 7133) (EC, 1997, updated 2003) and another opinion on the safety of *Enterococcus faecium* (DSM 7134) for piglets, pigs for fattening and sows (EC, 2003). EFSA published an opinion on the safety of Bonvital (*Enterococcus faecium* DSM 7134) for chickens for fattening (EFSA, 2004), an opinion on the safety and efficacy for piglets and pigs for fattening (EFSA, 2006) and an opinion on the safety and efficacy for sows (EFSA, 2007).

2. Proposed conditions of use

The product is intended for use in feed for chickens for fattening at a minimum content of 0.5 x 10^9 and a maximum content of 2.0 x 10^9 CFU kg^{-1} of complete feedingstuffs.[8]

2.1. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of the active agent in animal feeds. The Executive Summary of the CRL report can be found in the Appendix.

3. Efficacy

Four studies were provided with chickens for fattening (Ross 308), all of which were performed in a single European country but in three different locations. An additional field study was submitted and carried out in the same country. Those experiments included several doses of Bonvital, all confirmed by analysis.

The first trial was made with 4000 chickens for a period of 32 days.[9] The chickens were divided in four experimental groups and each experimental group was replicated in five pens of 200 chickens (100 males + 100 females). The four experimental groups were the control group (no supplementation) and three groups supplemented with Bonvital at 0.2 x 10^9, 1.0 x 10^9 or 2.0 x 10^9 CFU kg^{-1} feed.

The chickens fed Bonvital at 1.0 x 10^9 CFU kg^{-1} feed showed a significantly higher final body weight than the control group. The feed to gain ratio was significantly improved by 0.2 x 10^9

[8] The original minimum dose proposed by the Applicant was 0.2 x 10^9 CFU kg^{-1} of complete feedingstuffs which was then increased to 0.5 x 10^9 CFU kg^{-1} complete feed

[9] Technical dossier/Section III-1

and 1 x 10^9 CFU kg^{-1} feed (Table 2). The bacteriological profile in duodenum and caecum was obtained with ten chickens from each group and no differences were found. Mortality was not affected by treatment.

The second trial followed the same protocol as trial 1 but the duration was 33 days and the doses used in the treatment groups were 0.2 x 10^9, 0.5 x 10^9 or 1.0 x 10^9 CFU kg^{-1} feed.[10] The feed to gain ratio of chickens fed 0.5 x 10^9 CFU kg^{-1} feed was improved significantly in relation to the control group (Table 2). Other parameters were not significantly affected by treatment.

The third trial involved 2000 chickens and lasted 32 days.[11] The chickens were divided in two experimental groups. Each experimental group was replicated in five pens of 200 chickens (100 males + 100 females). The two experimental groups were the control group (no supplementation) and one group supplemented with Bonvital at 1.0 x 10^9 CFU kg^{-1} feed. No significant effect of treatment was observed (Table 2). In those three trials, the data were analysed using a fixed variance model and means were compared with Tukey's test.

The fourth trial in essence was a field trial made using two broiler houses from one farm.[12] One house was used for the control group (17500 birds) and the second house for the treatment group (17000 birds) supplemented with Bonvital at 0.5 x 10^9 CFU kg^{-1} feed. The duration of the study was 37 days. The final mean weight of chickens (80 birds per group randomly selected) from the Bonvital group was higher (+ 101 g) than in the control group (Table 2). The mortality rate was lower in the group supplemented with Bonvital than in the control group (501 vs. 703, $p < 0.05$). However, in the absence of a cross-over design no conclusions can be drawn on the effect of Bonvital.

The fifth trial involved only 100 birds divided in two experimental groups, a control group and a treatment group with Bonvital at 0.5 x 10^9 CFU kg^{-1} feed.[13] The duration of the study was 36 days. Individual body weights were measured at 15, 29 and 36 days. Feed intake was measured by group. The chickens fed with Bonvital showed a significant higher final body weight than in the control group (Table 2), but the effect cannot be traced back to the treatment because of potential pen interactions.

[10] Technical dossier/Section III-2
[11] Technical dossier/Section III-3
[12] Technical dossier/Section III-4
[13] Technical dossier/Section III-5

Table 2. Summary of the efficacy studies with Bonvital in chickens for fattening

Trial	Duration (days)	Dose (CFU kg^{-1} feed)	Final body weight (kg)	Feed intake (kg)	Feed/gain (kg kg^{-1})
1	32	0	1.40[a]	2.27	1.67[a]
		0.2×10^9	1.43[ab]	2.31	1.63[b]
		1.0×10^9	1.50[b]	2.39	1.63[b]
		2.0×10^9	1.46[ab]	2.33	1.64[ab]
2	33	0	1.61	2.72	1.72[a]
		0.2×10^9	1.62	2.70	1.70[ab]
		0.5×10^9	1.61	2.64	1.68[b]
		1.0×10^9	1.61	2.67	1.70[ab]
3	32	0	1.45	2.38	1.67
		1.0×10^9	1.48	2.38	1.65
4 (field trial)	37	0	2.02	-	1.52
		0.5×10^9	2.12		1.63
5	36	0	2.20[b]	3.49	1.58
		0.5×10^9	2.32[a]	3.62	1.56

[a, b]: Different superscripts in a column within a given trial indicate statistical difference ($P < 0.05$)

The FEEDAP Panel notes that efficacy was demonstrated around 0.5×10^9 CFU kg^{-1} feed in two studies. However, the FEEDAP Panel considers this insufficient to finally conclude on the efficacy of Bonvital at the minimum recommended dose.

3.1. Compatibility with coccidiostats

The compatibility of *Enterecoccus faecium* DSM 7134 when used in feed with coccidiostats has been studied by the applicant in an *in vitro* study.[14] The aim was to identify the minimum inhibitory concentration (MIC) of the coccidiostats robenidine hydrochloride, maduramicin ammonium, diclazuril, decoquinate, halofuginone hydrobromide, lasalocid A sodium and monensin sodium.

The MICs were measured in a broth dilution test. An overnight culture of strain of *E. faecium* DSM 7134 at 10^5 cells mL^{-1} was inoculated in brain-heart-media, containing increasing concentrations of different coccidiostats. After an incubation of 24 hours, the turbidity of the media was measured at 620 nm. Following international recognised standards, the MIC should be read as the concentration of the antimicrobial in which growth is reduced by 80 % or more as compared to the control. None of the analysed coccidiostats showed antimicrobial activity against the *E. faecium* DSM 7134 at the tested concentrations.

In reference to the EFSA Technical guidance on compatibility of microbial additives with substances showing antimicrobial activities (EFSA, 2008), robenidine hydrochloride, maduramicin ammonium, diclazuril, decoquinate and halofuginone hydrobromide lasalocid A sodium and monensin sodium showed compatibility at four times the feed use level with the Bonvital strain *E. faecium* DSM 7134.

4. Safety for the target animals

The safety of Bonvital for chickens for fattening was assessed in a previous opinion. Since then, however, the FEEDAP Panel has reconsidered its position with regard to tolerance studies and the need to follow completely the applicable Guidelines. The tolerance trial previously considered and the one newly submitted did not meet those requirements. Consequently, the FEEDAP Panel is unable to conclude on the safety of Bonvital for chickens for fattening.

[14] Technical dossier/Section IV

5. Post-market monitoring

Risks associated with the use of the product with chickens for fattening cannot be fully assessed.

CONCLUSIONS

The FEEDAP Panel notes that efficacy was demonstrated around 0.5 x 10^9 CFU kg^{-1} feed in two studies. However, the Panel considers this insufficient to finally conclude on the efficacy of Bonvital at the minimum recommended dose.

The compatibility of Bonvital has been demonstrated with the coccidiostats robenidine hydrochloride, maduramicin ammonium, diclazuril, decoquinate, halofuginone hydrobromide, monensin sodium and lasalocid A sodium.

The FEEDAP Panel is unable to conclude on the safety of Bonvital for chickens for fattening.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier for the application authorization of the feed additive Bonvital for animal category 'Chickens for fattening' according to Article 10 of Regulation (EC) No 1831/2003. February 2008. Submitted by Lactosan GmbH & Co. KG
2. Supplementary information on Bonvital for chickens, January 2009. Submitted by Lactosan GmbH & Co. KG
3. Evaluation report of the Community Reference Laboratory feed additives authorisation on the methods(s) of analysis for Bonvital (*Enterococcus faecium* DSM 7134) for chickens for fattening.
4. Amendment to CRL Report (D08/FSQ/CVH/RL/D(2008)26412) on the dossier EFSA-Q-2008-289 (Bonvital) of 3 February 2009.
5. Comments from the Member States submitted through the ScienceNet.

REFERENCES

EC (European Commission), 1997, updated 2003. Opinion on the use of certain micro-organisms as additives in feedingstuffs. <http://ec.europa.eu/food/fs/sc/scan/out93_en.pdf>

EC (European Commission), 2003. Report of the Scientific Committee on Animal Nutrition on the safety of the Micro-organism product Provita E® for use as feed additive. <http://ec.europa.eu/food/fs/sc/scan/out107_en.pdf>

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the safety of product Bonvital (Provita E) for chickens for fattening. <http://www.efsa.europa.eu/en/science/feedap/feedap_opinions/693.html>

EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety and efficacy of product Bonvital, a preparation of *Enterococcus faecium*, as a feed additive for piglets and pigs for fattening in accordance with Regulation (EC) No 1831/2003. <http://www.efsa.europa.eu/en/science/feedap/feedap_opinions/ej440_bonvital.html>

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) on the safety and efficacy of

product Bonvital, a preparation of *Enterococcus faecium*, as a feed additive for sows in accordance with Regulation (EC) No 1831/2003. <http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178622724657.htm>

EFSA (European Food Safety Authority), 2008. Technical guidance. Compatibility of zootechnical microbial additives with other additives showing antimicrobial activity. Prepared by the Panel on Additives and Products or Substances used ion Animal Feed. <http://www.efsa.europa.eu/cs/BlobServer/Scientific_Document/feedap_guidance_ej658_compatibility_en,0.pdf?ssbinary=true>

APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for Bonvital

In the current application authorisation is sought for the microbial feed additive Bonvital under the category 'zootechnical additives', functional group 'gut flora stabilisers' according to Annex I of Regulation (EC) No 1831/2003. The active agent in the additive is *Enterococcus faecium* DSM 7134. The additive is available in two forms (powder or granules (micro-encapsulated)) both of which contain a minimum concentration of 1×10^{10} colony forming units (CFU) per gram. Specifically, authorisation is sought to use Bonvital for chickens for fattening. The conditions of use are proposed with a recommended dosage of 0.2 to 2.0×10^{9} CFU/kg.

For the quantification of the active agent (*Enterococcus faecium* DSM 7134) of Bonvital in the feed additive, premixtures and feedingstuffs, an appropriate pour plate method using a selective enterococci agar was proposed by the applicant. The method was in-house validated and was shown to be transferable to three external laboratories. The method precision data resulting from the in-house and between-laboratory trials were acceptable for the intended purpose.

For official controls regarding the quantitative determination of the colony forming units of the active agent in the feed additive, premixtures and feedingstuffs, a spread plate enumeration method is recommended which has been fully ring-trial validated (J. Appl. Microbiol. 2002, 93, 781-786).

The method's performance characteristics of the enumeration method are standard deviations for repeatability (sr) and reproducibility (sR) of around 0.12 – 0.20 $\log_{10}$ and 0.23 – 0.41 $\log_{10}$ calculated from the base 10 logarithms of the measured CFU/g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around 104 colony forming units (CFU) per gram (g) feed additive or premixture and around 10^{7} CFU/kg feedingstuff.

The identity of the bacterial strain, *Enterococcus faecium* DSM 7134, was analysed by a range of techniques including biochemistry, protein-fingerprinting and molecular methods such as polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE). PFGE is a generally recognised standard methodology for microbial identification and is considered suitable for official controls in the frame of the authorisation.

On the basis of the supplied documentation, no supplementary experimental work (testing or method validation) is required.

APPENDIX B

Amendment to CRL Report (D08/FSQ/CVH/RL/D(2008)26412) on the dossier EFSA-Q-2008-289 (Bonvital)

Based on the proposed register entry the CRL-FA recommend in the above-mentioned report a fully ring trial validated spread plate enumeration method (J. Appl. Microbiol. 2002, 93, 781-786) for the quantitative determination of the colony forming units of the active agent in feedingstuffs, to be used within the frame of official controls. With the present amendment the CRL-FA confirms the validity of this statement also for the modified minimum content of the active agent in feed, i.e. 0.5×10^9 instead of 0.2×10^9 CFU/kg feed.