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SCIENTIFIC OPINION

Compatibility of the microbial product 035 (*Bacillus subtilis*) with decoquinate and narasin/nicarbazin[1]

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

(Question No EFSA-Q-2008-423)

Adopted on 22 October 2008

PANEL MEMBERS

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a scientific opinion on the compatibility of the microbial product 035 (*Bacillus subtilis*) with decoquinate and narasin/nicarbazin.

A 35-day compatibility trial with 450 one-day-old male chickens divided into one control and four test groups was performed. The birds received the recommended dose of 035 (8×10^8 CFU kg^{-1}, confirmed by microbiological analysis) and each of the four experimental groups, one of the following coccidiostats: decoquinate, robenidine, diclazuril or narasin/nicarbazin, at the highest authorised dose levels. The birds were observed for mortality and morbidity, body weight gain and feed conversion. On day 14, one bird from every cage was randomly selected and analysed for the caecal content of bacilli. At the end of the trial the analyses were repeated using three birds per cage, the total number of analysed birds being 180.

There were no statistically significant differences between the groups in the weight gain or feed to gain ratio. The presence of the coccidiostats decoquinate or narasin/nicarbazin did not significantly reduce *Bacillus* counts. Given the number of replicates, those results are considered reliable and allow the conclusion that 035 is compatible *in vivo* with decoquinate and narasin/nicarbazin when used at the authorised levels.

The study also included matching data on the coccidiostats robenidine and diclazuril. The results confirm the compatibility of 035 with those two coccidostats.

[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 compatibility of the microbial product 035 (*Bacillus subtilis*) with decoquinate and narasin/nicarbazin. *The EFSA Journal* (2008) 840, 1-7

Key words: zootechnical additive, gut flora stabilisers, 035, *Bacillus subtilis*, chickens for fattening, compatibility, decoquinate, narasin/nicarbazin

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BACKGROUND

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additives for animal nutrition and in particular defines the conditions that a substance/product should meet to be granted the authorisation. This Regulation replaces Council Directive 70/524/EEC.

The European Commission received a request from Chr. Hansen A/S[2] for authorisation of use the product 035 (*Bacillus subtilis* DSM 17299) to be used as a feed additive for chickens for fattening, containing the coccidiostats decoquinate and narasin/nicarbazin.

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 13(3) (modification of the authorisation of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[3] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment on the proposals for change made by the applicant. The particulars and documents in support of the application were considered valid by EFSA as of 8 September 2008.

TERMS OF REFERENCE

In view of the above, the Commission asks to the European Food Safety Authority to deliver an opinion on the compatibility of the microbial preparation 035 (*Bacillus subtilis* DSM 17299) with the coccidiostats decoquinate and narasin/nicarbazin, 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] Chr. Hansen, Boege Allé, 10-12, DK-2970, Hoersholm, Denamrk
[3] Dossier reference: FAD-2008-0019

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

Additive	*Bacillus subtilis* DSM 17299
Registration number/EC No/No (if appropriate)	4b1821
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)**
Min.1.6 x 10^9 CFU/g of *Bacillus subtilis* DSM 17299. Whey permeate or limestone as carrier.	Not applicable	Complies with EU legislation on microbial quality and undesirable substance	Microbial counting technique based on plating on Tryptose Blood Agar, TBA (later on Tryptic Soya Agar, TSA)

Trade name (if appropriate)	035, also called GalliPro®
Name of the holder of authorisation (if appropriate)	Chr. Hansen A/S, Denmark

Conditions of use				
Species or category of animal	**Maximum Age**	**Minimum content**	**Maximum content**	**Withdrawal period (if appropriate)**
		CFU kg^{-1} of complete feedingstuffs		
Chickens for fattening	Slaughter age	0.8 x 10^9	1.6 x 10^9	Not applicable

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	None
Specific conditions or restrictions for handling (if appropriate)	Face mask, gloves and goggles should be worn when handling the product to reduce contact with dust.
Post market monitoring (if appropriate)	PMM is included in the general handling system of incoming complaints. Qualified employees will be handling each reported case, and evaluate if any adverse effects are seen by the use of the product, and take the needed actions. Data and samples of each produced batch are kept for at least five years if further tests should be needed. The production sites producing 035 are GMP, Famiqs and/or ISO certified which includes the principles of HACCP.
Specific conditions for use in complementary feedingstuffs (if appropriate)	Dosage in final complete feedingstuff should be 0.8-1.6 x 10^9 CFU per kg. Can be used in feedingstuff containing the coccidiostats **decoquinate**, diclazuril, halofuginone, *lasalocid sodium, maduramicin ammonium, monensin sodium, narasin*, **narasin/nicarbazin**, robenidine, *salinomycin sodium and semduramicin sodium.*

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	**Species or category of animal**	**Target tissue(s) or food products**	**Maximum content in tissues**
Not applicable	Not applicable	Not applicable	Not applicable

ASSESSMENT

1. Introduction

The additive 035 is a microbial feed additive based on *Bacillus subtilis* (DSM 17299). In its previous opinions, the FEEDAP Panel was unable to reach a conclusion on the compatibility of this microbial product with the coccidiostats lasalocid sodium, maduramicin ammonium, monensin sodium, narasin, salinomycin sodium and semduramicin sodium, based on the data available. The product is currently authorised to be used in feed containing robenidine, halofuginone and diclazuril. The applicant has now provided new data to support the compatibility of 035 with decoquinate and narasin/nicarbazin.

2. Compatibility of 035 with coccidiostats

A 35-day compatibility trial was performed with 450 one-day-old male chickens divided into control and four test groups, each consisting of 90 birds (nine cages of ten birds per group). The birds received the authorised dose of 035 (8×10^8 CFU kg^{-1} confirmed by microbiological analysis), and each of the four experimental groups, one of the following coccidiostats: decoquinate (40 mg kg^{-1}), robenidine (66 mg kg^{-1}), diclazuril (1 mg kg^{-1}) or narasin/nicarbazin (80 – 100 mg kg^{-1}). The birds were observed for mortality and morbidity, body weight gain and feed conversion. On day 14, one bird from every cage was randomly selected and euthanised, and the caecal contents were collected to analyse the bacilli content. At the end of the trial, the analyses were repeated using three birds per cage, the total number of analysed birds being 180.

The microbiological analysis were performed by comparing the plate counts of samples with and without a heat treatment (80 °C water bath for ten minutes) to differentiate between heat resistant spores and vegetative cells.

There were no statistically significant differences between the groups in the weight gain or feed conversion ratio (Table 2). The 35-day caecal *Bacillus* counts (vegetative cells and spores) were similar, irrespective of the coccidiostat addition (Table 2).

Table 2. **Average weight gain, feed to gain ratio and caecal *Bacillus* counts of broilers used in the 35-day compatibility trial**

	Average weight gain (kg)	Feed/gain ratio (kg kg^{-1})	*Bacillus* counts (log CFU g^{-1})[1]	
			Heat treated	Non heat treated
Control	2.13	1.55	5.19	5.16
+ decoquinate	2.12	1.54	4.92	4.95
+ robenidine	2.11	1.55	5.12	5.15
+ diclazuril	2.07	1.61	5.21	5.25
+ narasin/nicarbazin	2.07	1.54	5.10	5.10

[1]Heat treated: spores counts; non-heat treated: spore + vegetative cell counts

When the CFU counts of heat-treated and non-heat-treated samples were compared, the average numbers were 1.32×10^5 CFU g^{-1} and 1.36×10^5 CFU g^{-1}, respectively. In one-tailed Student's t-test, this difference was found non-significant ($P = 0.43$).

CONCLUSIONS

The presence of the coccidiostats decoquinate or narasin/nicarbazin did not significantly reduce *Bacillus* counts in the caecum. Given the number of replicates, those results are considered reliable and allow the conclusion that 035 is compatible *in vivo* with decoquinate and narasin/nicarbazin when used at the authorised levels.

The study also included matching data on the coccidiostats robenidine and diclazuril. The results confirm the compatibility of 035 with those two coccidiostats.

Remark

The Register entry as proposed by the applicant, under specific conditions of use does not reflect the authorised/applied status of compatibility with coccidiostats.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier with supplementary information on the zootechnical feed additive "035" for chickens for fattening, and the use of the product in feed containing two coccidiostats. May 2008. Submitted by Chr. Hansen A/S.