

SCIENTIFIC OPINION

Scientific Opinion on the compatibility of the microbial product 035 (*Bacillus subtilis*) with lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium¹

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{2,3}

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver an opinion on the compatibility of microbial feed additive 035 based on *Bacillus subtilis* (DSM 17299) with the coccidiostats lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium. While the compatibility of *Bacillus subtilis* (DSM 17299) with lasalocid sodium has been established in a previous assessment of the product BioPlus 2B containing the same strain, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was unable to conclude on the compatibility with the rest of the coccidiostats. The applicant has now provided new data to support the compatibility of the product 035 with the coccidiostats mentioned above.

Two studies have been provided, involving 560 and 600 chickens for a period of 34 and 28 days, respectively. The first trial was performed with the product BioPlus 2B, while the second one was done with 035. In both cases, the birds received a basal diet containing *B. subtilis* DSM 17299 at the recommended inclusion rate in the presence or absence of the coccidiostats monensin sodium, salinomycin sodium and semduramycin sodium (trial 1) or salinomycin sodium, narasin, monensin sodium and maduramycin ammonium (trial 2) at their maximum authorised levels. At the end of the experiments caecal samples were randomly collected and analysed for *B. subtilis* applying a heat treatment to distinguish between the vegetative cells and spores. No significant differences in the CFU counts were observed between the control and the treated groups in any of the two trials.

The FEEDAP Panel concludes that the product 035 (*B. subtilis* DSM 17299) is compatible with lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium.

¹ On request from the European Commission, Question No EFSA-Q-2009-00803, adopted on 10 March 2010.

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³ Acknowledgement: The Panel wishes to thank the members of the Working Group on Micro-organisms, including Atte von Wright and Guido Rychen, for the preparation of this opinion.

KEY WORDS

Zootechnical additive, *Bacillus subtilis*, chickens for fattening, compatibility, coccidiostats, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium, semduramycin sodium

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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. Regulation foresees also the possibility to modify authorisations already given in its Article 13. The company Chr. Hansen A/S is seeking to modify the current Community authorisations of its product 035, a microbial preparation of *Bacillus subtilis* DSM 17299, as regards its compatibility with some coccidiostats. Notably, the Company is asking to assess the compatibility of 035 with lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium.

The EFSA in its opinion adopted on 14 May 2009 (Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the microbiological product "035", a preparation of *Bacillus subtilis* DSM 17299 as a feed additive for chickens for fattening in accordance with Regulation (EC) 1831/2003) was not able to give a conclusive opinion on the compatibility of the product with some coccidiostats because of lack of data provided by the Company. EFSA had assessed this issue earlier in its opinions of 17 October 2006 (Opinion of the Scientific Panel on Additive and Products or Substance used in Animal Feed on the microbiological product "035", a preparation of *Bacillus subtilis* DSM 17299 as a feed additive for chickens for fattening in accordance with Regulation (EC) 1831/2003) and 18 October 2007 (Opinion of the Panel on additives and products or substances used in animal feed (FEEDAP) on the compatibility of the microbial product 035, a preparation of *Bacillus subtilis* with lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium).

Therefore, the Commission gave the possibility to the company to submit complementary information to complete these assessments. The Commission has received a supplementary dossier⁴ from the applicant company Chr. Hansen A/S containing new data to support the compatibility of 035 with lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium. The data generated by the company and compiled in the above mentioned supplementary dossier have been sent directly to the Authority.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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: lasalocid sodium, maduramycin ammonium, monensin sodium, narasin, salinomycin sodium, and semduramycin sodium, under the requested conditions of use.

⁴ EFSA dossier reference: FAD-2009-0042

ASSESSMENT

1. Introduction

The additive 035 is a microbial feed additive based on *Bacillus subtilis* (DSM 17299). The additive is intended to be used in feeds for chickens for fattening at the minimum dose of 8×10^8 and a maximum of 1.6×10^9 CFU/kg complete feedingstuffs.

The compatibility of 035 with the coccidiostats decoquinate, narasin/nicarbazin, robenidine and diclazuril when added to chickens' diets has been established in a previous opinion (EFSA, 2008a). The compatibility of *Bacillus subtilis* DSM 17299⁵ with lasalocid sodium has been established in connection with a compatibility study with the product BioPlus 2B from the same company and containing this strain (EFSA, 2008b). In other opinions, however, the FEEDAP Panel was unable to reach a conclusion on the compatibility of the product 035 with the coccidiostats maduramycin ammonium, monensin sodium, narasin, salinomycin sodium and semduramycin sodium, based on the data available (EFSA, 2006a, 2007 and 2009).

The applicant has now provided new data to support the compatibility of 035 with those five coccidiostats.

2. Compatibility of 035 with coccidiostats

2.1. Compatibility of 035 with monensin sodium, salinomycin sodium and semduramycin sodium

An experiment was performed with the feed additive BioPlus 2B, which contains a 1:1 mixture of *Bacillus subtilis* DSM 17299 and *B. licheniformis* DSM 5749. The FEEDAP Panel considers this approach acceptable for the assessment of 035 since identification at strain level was performed. The differentiation of the two bacillar species was based on colony morphology and confirmed by Pulsed Field Gel Electrophoresis. The method has been verified by the Community Reference Laboratory (CRL), as stated in the EFSA opinion on the compatibility of BioPlus 2B with maduramycin ammonium ammonium (EFSA, 2006b).

A total of 560 one-day-old chickens were distributed into four treatments (four pens of 35 birds per treatment). The birds were fed for 34 days the basal feed containing BioPlus 2B at a dose ensuring content of *B. subtilis* DSM 17299 at the minimum recommended dose (8×10^8 CFU/kg feed, confirmed by analysis), with or without monensin sodium (128 mg/kg), salinomycin sodium (65.8 mg/kg) and semduramycin sodium (30.9 mg/kg).⁶ The doses of coccidiostats are close to their respective maximum authorised levels (125, 70 and 25 mg/kg, respectively). The birds were followed for weight gain and feed to gain ratio. At the end of the trial, caecal samples were collected from 20 birds per treatment and analysed for *B. subtilis*. The analysis was performed with and without heat treatment in order to differentiate between the vegetative cells and spores. The results are given in Table 1.

Despite the poor performances observed, the experiment allows the assessment of the compatibility of *B. subtilis* with the three considered coccidiostats. There were no significant differences between the control and the treated groups in caecal *B. subtilis* counts, which were not affected by the heat treatment. There was no control group without BioPlus 2B supplementation, but in a previous opinion on 035 for chickens for fattening (EFSA, 2006a) the background levels of aerobic spore formers were found to be negligible in comparison to the levels found in treated birds.

⁵ Also registered with the deposition number DSM 5750.

⁶ Technical dossier/Final report incl. signature.

Table 1: Caecal *Bacillus subtilis* counts from chickens (34 days) treated with monensin sodium, salinomycin sodium and semduramycin sodium

| Treatment | Log CFU <i>Bacillus subtilis</i> ± SD/g caecal content | |
|---------------------|--|------------------|
| | + Heat treatment | - Heat treatment |
| Control | 4.15 ± 0.24 | 4.29 ± 0.42 |
| Monensin sodium | 4.10 ± 0.28 | 4.24 ± 0.42 |
| Salinomycin sodium | 4.13 ± 0.24 | 4.35 ± 0.23 |
| Semduramycin sodium | 4.21 ± 0.34 | 4.19 ± 0.27 |

2.2. Compatibility of 035 with salinomycin sodium, narasin, monensin sodium and maduramycin ammonium

In the context of a former opinion, a 35-day study carried out with 600 one-day-old chickens was assessed (EFSA, 2009). The birds received 035 at the recommended inclusion rate (8×10^8 CFU/kg feedingstuff, confirmed by analysis) and were divided into six treatment groups, each consisting of three replicates (pens) of 40 birds. One group served as the control while each of the other groups received one of the following coccidiostats at the maximum authorised level: salinomycin sodium (70 mg/kg), narasin (70 mg/kg), monensin sodium (125 mg/kg) and maduramycin ammonium (5 mg/kg). The birds were followed for performance throughout the starter, grower and finisher phase. At the end of the grower phase (day 28), caecal samples were randomly collected (six birds per pen) and analysed for *Bacillus* counts. The medium contained colistine sulphate to reduce the background growth. While no significant differences in the caecal *Bacillus* counts were detected, the FEEDAP Panel could not conclude on the compatibility of *B. subtilis* DSM 17299 with the coccidiostats included since no treatment to distinguish the vegetative and spore forms had been applied.

The applicant has now completed the data with caecal spore counts (16–18 caecal samples per treatment) from both heat treated and non-heat treated samples. The results are given in Table 2. No significant differences in the CFU counts were observed between the treatments groups or between the heat treated and non-heat treated samples.⁷

Table 2: Caecal *Bacillus subtilis* counts from chickens treated with salinomycin sodium, narasin, monensin sodium or maduramycin ammonium

| Treatment | Log CFU <i>Bacillus subtilis</i> ±SD/g caecal content | |
|----------------------|---|--------------------|
| | + Heat treatment | - Heat treatment |
| Control | 5.23 ± 0.30 (n 18) | 5.46 ± 0.49 (n 17) |
| Salinomycin sodium | 5.17 ± 0.38 (n 17) | 5.28 ± 0.48 (n 16) |
| Narasin | 5.11 ± 0.37 (n 18) | 5.47 ± 0.33 (n 18) |
| Monensin sodium | 5.10 ± 0.45 (n 18) | 5.39 ± 0.36 (n 16) |
| Maduramycin ammonium | 5.19 ± 0.29 (n 18) | 5.42 ± 0.37 (n 16) |

CONCLUSIONS

The treatment of chickens for fattening with the highest authorised doses of either maduramycin ammonium, monensin sodium, narasin, salinomycin sodium or semduramycin sodium did not affect the caecal CFU counts of *B. subtilis* DSM 17299, when added at the minimum recommended dose. Since the CFU analysis took into account both spores and vegetative cells, the FEEDAP Panel concludes that 035 is compatible with those coccidiostats.

As established in a previous opinion, *B. subtilis* DSM 17299 is also compatible with lasalocid sodium.

⁷ Technical dossier/Technical Report TR04181 and Appendix to TR04181

DOCUMENTATION PROVIDED TO EFSA

1. Compatibility study with GalliPro and ionophore coccidiostats for chickens. September 2009. Submitted by Chr. Hansen A/S.
2. Supplementary dossier on compatibility study with BioPlus 2B and coccidiostats and preservatives used for poultry feed. November 2009. Submitted by Chr. Hansen A/S.

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