SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of the product Cylactin® (Enterococcus faecium) as a feed additive for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

ABSTRACT

Cylactin® is a product consisting of viable cells of Enterococcus faecium. The product strain was found to be resistant to kanamycin. A detailed study of the nature of kanamycin resistance demonstrated the absence of known acquired genes coding for aminoglycosides-modifying enzymes. Two genetic determinants, the aminoglycoside acetyltransferase gene aac(6')-li and the gene sfkm, both intrinsic to E. faecium, were shown to contribute to the resistance to kanamycin. In the view of the FEEDAP Panel, the resistance of this strain of E. faecium is most likely to be caused by an unknown mechanism that potentiates the effect of the sfkm gene, and not by acquisition of resistance genes, and thus is not a cause for concern. When used at 100 times the recommended level, Cylactin® did not show any adverse effect on performance or mortality of chickens for fattening. Therefore, the additive is safe for the target animals at the proposed conditions of use. E. faecium NCIMB 10415 is free of known enterococcal virulence factors. No other concerns for consumer safety were identified. Cylactin® is not a skin/mucosal irritant or skin sensitiser. As the product is formulated with a large particle size and the dusting potential is low, inhalation exposure of users will be minimal. Cylactin® is efficacious in chickens for fattening as demonstrated in three trials showing an increase in final body weight when diets were supplemented at or close to the minimum recommended dose (0.3 x 10^9 CFU/kg feed). Cylactin® is compatible with decoquinate, monensin, robenidine, diclazuril and semduramycin.

KEY WORDS

Zootechnical additive, gut flora stabilisers, Cylactin®, Enterococcus faecium, kanamycin resistance, chickens for fattening, safety, efficacy, compatibility.
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 Cylactin® (Enterococcus faecium) as a feed additive for chickens for fattening.

The microbial feed additive Cylactin® is a product consisting of viable cells of a Enterococcus faecium. The product strain was found to be resistant to kanamycin. A detailed study of the nature of kanamycin resistance, demonstrated the absence of known acquired genes coding for aminoglycosides-modifying enzymes. Two genetic determinants, the aminoglycoside acetyltransferase gene aac(6’)-li and the gene sfkm’, both intrinsic to E. faecium, were shown to contribute to the resistance to kanamycin. In the view of the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), the resistance of this strain of E. faecium is most likely to be caused by an unknown mechanism that potentiates the effect of the sfkm’ gene, and not by acquisition of resistance genes, and thus is not a cause for concern.

When used at 100 times the recommended dose, Cylactin® did not show any adverse effect on the performance or mortality of chickens for fattening. Therefore, the additive is safe for the target animals at the proposed conditions of use.

Enterococcus faecium NCIMB 10415 is free of known enterococcal virulence factors. No other concerns for consumer safety were identified.

Cylactin® is not a skin/mucosal irritant or skin sensitiser. As the product is essentially dust-free, inhalation exposure of users will be minimal.

Cylactin® is efficacious in chickens for fattening as demonstrated in four trials showing an increase in final body weight when diets were supplemented at or close to the minimum recommended dose (0.3 x 10⁹ CFU/kg feed). Cylactin® is compatible with the coccidiostats decoquinate, monensin, robenidine, diclazuril and semduramycin.
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BACKGROUND

Regulation (EC) No 1831/2003\(^4\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation lays down that an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of the Regulation (EC) No 1831/2003 for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company DSM Nutritional products Sp. Z.o.o.\(^5\) for authorisation of the product Cylactin\(^6\), \textit{Enterococcus faecium} NCIMB 10415, to be used as a feed additive for chickens for fattening (category: zootechnical additive; functional group: gut flora stabiliser) 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 10(2) (re-evaluation of an authorised additive). EFSA received directly from the applicant the technical dossier in support of this application.\(^7\) 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 27 October 2008.

The Scientific Committee on Animal Nutrition issued on the safety for the target animals, consumers, users and environment for this product when used as a feed additive for chickens for fattening, piglets, pigs for fattening and calves (EC, 1997, updated 2003).

EFSA issued one opinion on the safety of Cylactin\(^8\) (\textit{Enterococcus faecium} NCIMB 10415) for dogs and cats (EFSA, 2004).

The product \textit{Enterococcus faecium} NCIMB 10415 has been authorised for chickens and pigs for fattening,\(^8\) piglets,\(^9\) sows,\(^10\) calves,\(^11\) turkeys for fattening,\(^12\) and cats and dogs.\(^13\)

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, user and the environment of the product Cylactin\(^8\), \textit{Enterococcus faecium} NCIMB 10415, when used under the conditions described in Table 1.

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\(^4\) OJ L 268, 18.10.2003, p. 29.
\(^5\) DSM Nutritional products Sp. Z.o.o., 96-320 Mszczonow, Poland.
\(^6\) The Applicant intends to market the product also under the tradename Cernivet\(^8\) LBC ME 10 and 20.
\(^7\) EFSA Dossier reference: FAD-2008-0021.
\(^10\) OJ L 44, 15.2.2006, p. 44.
\(^11\) OJ L 243, 15.7.2004, p. 10;
\(^12\) OJ L 335, 20.12.2007, p. 17.
\(^13\) OJ L 34, 4.2.2009, p. 8.
### Table 1: Description and conditions of use of the additive as proposed by the applicant

<table>
<thead>
<tr>
<th>Additive</th>
<th>Enterococcus faecium NCIMB 10415</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration number/EC No</td>
<td>E 1705</td>
</tr>
<tr>
<td>Category of additive</td>
<td>Zootechnical additive</td>
</tr>
<tr>
<td>Functional group of additive</td>
<td>Gut flora stabiliser</td>
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</tbody>
</table>

#### Description

<table>
<thead>
<tr>
<th>Composition, description</th>
<th>Chemical formula</th>
<th>Purity criteria</th>
<th>Method of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Colony forming unit</td>
</tr>
</tbody>
</table>

#### Trade name

- Cylactin®
- Cernivet®

#### Name of the holder of authorisation

DSM Nutritional Products Ltd

#### Conditions of use

<table>
<thead>
<tr>
<th>Species or category of animal</th>
<th>Maximum Age</th>
<th>Minimum content</th>
<th>Maximum content</th>
<th>Withdrawal period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens for fattening</td>
<td>No limitation</td>
<td>0.3 x 10⁹</td>
<td>2.8 x 10⁹</td>
<td>None</td>
</tr>
</tbody>
</table>

#### Other provisions and additional requirements for the labelling

- Specific conditions or restrictions for use: None
- Specific conditions or restrictions for handling: *E. faecium* NCIMB is a class 1 biological agent. No specific labelling required.
- Post-market monitoring: No additional requirements further to the need for traceability and recall procedures established by Regulation No 178/2002.
- Specific conditions for use in complementary feedingstuffs: None

#### Maximum Residue Limit (MRL)

<table>
<thead>
<tr>
<th>Marker residue</th>
<th>Species or category of animal</th>
<th>Target tissue(s) or food products</th>
<th>Maximum content in tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Not relevant</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>
ASSESSMENT

1. Introduction

The microbial feed additive Cylactin® is a product consisting of dehydrated cells of *Enterococcus faecium* NCIMB 10415. The product has already been granted permanent authorisation for use in piglets up to 35 kg, pigs and chickens for fattening, sows from two weeks prior to farrowing and during lactation, calves, and cats and dogs. The applicant is now seeking a re-evaluation of the use of *Enterococcus faecium* NCIMB 10415 in diets for chickens for fattening at an inclusion level of 0.3 x 10^9–2.8 x 10^9 CFU/kg complete feedingstuff.

2. Characterisation

2.1. The nature of the additive

This feed additive is a free flowing granulate available under two forms:

- **Cylactin® LBC ME10**, with a guaranteed minimal concentration of micro-encapsulated *E. faecium* NCIMB 10415 in the additive of 1 x 10^10 CFU/g, in a carrier composed by saccharose and hydroxypropylecullulose.

- **Cylactin® LBC ME20 plus**, with a guaranteed minimal concentration of micro-encapsulated *E. faecium* NCIMB 10415 in the additive of 2 x 10^10 CFU/g. In this formulation, the content of hydroxypropylecullulose is partially replaced by a resin.

Analyses of three batches of the product LBC ME10 showed that there are no particles below 100 µm of diameter, while 1–5 % of the product shows a particle size between 100 and 400 µm. Analyses of two batches of the product LBC ME20 showed that there were no particles below 100 µm of diameter. The dusting potential measured in three batches (two for the LBC ME20 form) was seen to be in average between 1–3.7 mg/m³, indicating that both products can be considered as practically dust free. No clear data on batch to batch variation was provided but evidence of compliance of five batches with the minimum concentration of the active agent.

HACCP procedures are specified and all raw materials checked for impurities. Analytical data from three batches showed concentrations of heavy metals (Pb, Hg, Cd), As and mycotoxins (aflatoxins B1, B2, G1, G2 and ochratoxin A) well below the specified concentrations at which corrective actions would be required. Numbers of clostridia, *Salmonella*, *E. coli* and other microbial contaminants were below detection levels.

2.2. Characterisation of the active agent

The active agent of the product is *E. faecium* SF68, isolated from faeces of a healthy newborn baby and deposited in the National Collection of Industrial, Marine and Food Bacteria (NCIMB), UK, with deposition number NCIMB 10415. The strain has not been genetically modified.

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14 The Applicant intends to market the product also under the tradename Cernivet® LBC ME 10 and 20 plus.
15 This section has been edited following the provisions of Article 18 of Regulation (EC) No 1831/2003.
16 Technical dossier/Supplementary information, Dec 09/Appendix 4-7.
17 Technical dossier/Section II/Appendix 2-15.
18 Technical dossier/Section II/Appendices 2-16 and 2-17.
19 Technical dossier/Section II/Appendices 2-5 and 2-6.
20 Technical dossier/Section II/Appendices 2-5, 2-6, 2-11 and 2-12.
21 Technical dossier/Section II/Appendix 2-18.
The strain has been identified by phenotypic tests and molecular taxonomy as *E. faecium* and characterised by PCR fingerprinting methods. The genetic stability of the strain has been assessed by analysing DNA fingerprints and metabolic properties.

### 2.2.1. Virulence

*E. faecium* NCIMB 10415 was demonstrated to be free of the putative enterococcal virulence factors aggregation substances, haemolysin, adhesin *Ace1* and *esp*, gelatinase and hyaluronidase.

### 2.2.2. Antimicrobial resistance

The susceptibility of the production strain to the antibiotics recommended by the FEEDAP Panel in its Technical Guidance on the update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance (EFSA, 2008a) was tested by a dilution method. The MICs of *E. faecium* NCIMB 10415 were lower than the EFSA breakpoints except for erythromycin (8 mg/L vs. 4 mg/L) and kanamycin (2048 mg/L vs. 512 mg/L). The low MIC value for erythromycin does not raise concern for the safety of *E. faecium* NCIMB 10415. However, the resistance to kanamycin is of greater concern.

Kanamycin Minimal Inhibitory Concentrations (MICs) in *E. faecium* show a bimodal distribution which is typical of acquired resistances. Most of the strains have an intrinsic low level resistance (MIC < 512 mg/L) while a limited percentage of strains (8.1 %, NARMS, 2007) present a level of resistance greater than 1024 mg/L. High-level aminoglycoside resistance in enterococci is mediated by aminoglycoside-modifying enzymes and thus is not intrinsic in *E. faecium*.

The applicant provided the complete genome sequence of *E. faecium* NCIMB 10415 demonstrating the absence of the known acquired aminoglycosides resistance genes (*aac(6')-le-aph(2'')-Ia, aph(2')-Ib, aph(2')-Ic, aph(2')-Id, aph(2')-Ie, aph(3')-IIIa, ant(4')-Ia*), other than aminoglycoside acetyltransferase gene *aac(6')-Ia*, which codes for intrinsic low level aminoglycoside resistance in *E. faecium*. However, the presence of this gene does not account for the unusually high resistance to kanamycin found in this strain.

Resistance to kanamycin was found to be associated with the presence of a novel described gene *sfkmr*. The functionality of the *sfkmr* gene, coding for a putative rRNA methylase, has also been demonstrated in a heterologous host (*Escherichia coli*). A partial inactivation of the gene reduced the resistance to kanamycin (MIC < 512 mg/L). The gene *sfkmr* was found to be widely distributed within *E. faecium*. However, *sfkmr* does not usually confer high resistance to kanamycin.

The FEEDAP Panel concludes that the resistance to kanamycin shown in this strain is most likely to be caused by an unknown mechanism that potentiates the effect of the *sfkmr* gene and not by acquisition of genes coding for aminoglycosides-modifying enzymes.

### 2.3. Manufacturing process

Cylactin® is produced by fermentation. Cells are recovered by filtration and washed prior to drying. The whole fermentation and purification processes are carried out under aseptic conditions to avoid contamination.
2.4. Stability and homogeneity

Stability tests to establish the shelf-life of Cylactin® have been performed over a 12-month period on three batches of LBC ME20 at 2–8°C, and for six months at 25 °C for both forms. Microbiological analyses provide evidence of the stability of the product when retained in closed polyethylene containers.  

Resistance to pelleting at temperatures up to 95 °C were tested in five and four batches for LBC ME10 and LBC ME20, respectively. One form of the product (LBC ME 10) was found to be stable up to 75 °C, while the other (LBC ME20) up to 85 °C.

The stability of Cylactin® LBC ME20 was tested in typical premixtures containing trace elements over four months. Microbiological analyses on two batches provide evidence of stability over a period of three months.

Studies on stability in complete feeds (mash and pelleted) were performed with two batches of each form of the product for three months (temperature from 15 °C to 25 °C). Microbiological analyses provided evidence of stability over a period of three months.

The homogeneity of LBC ME10 in mixtures was assessed in three batches of a non-specified feed. Tests of LBC ME20 were carried out with one batch of a premixture for chickens and one of a swine pelleted feed. The analysis of ten sub-samples gave a coefficient of variation of 7–22 %.

2.5. Compatibility with coccidiostats

The compatibility of *E. faecium* NCIMB 10415 with decoquinate, monensin, robenidine, lasalocid, narasin, salinomycin, maduramycin, diclazuril, narasin/nicarbazin and semduramycin has been evaluated according to the FEEDAP Technical guidance on the compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA, 2008b). The results showed that for the following coccidiostats the respective MICs were higher than four times the maximum authorised levels in feed: decoquinate, monensin, robenidine, diclazuril and semduramycin. Compatibility has been demonstrated for decoquinate, monensin, robenidine, diclazuril and semduramycin.

The remaining coccidiostats showed MICs lower than four times the maximum authorised levels in feed. Consequently, a four-week experiment was conducted to evaluate the in vivo compatibility between Cylactin® LBC ME10 and various coccidiostats. A total of 80 one-day-old chickens (Cobb, 40 males and 40 females) were distributed in 40 cages and fed standard starter and grower diets. The following seven treatments were applied: i) negative control (no Cylactin®), no coccidiostats, ii) Cylactin® at 0.3 x 10⁹ CFU/kg, no coccidiostats, iii) Cylactin® at 0.3 x 10⁹ CFU/kg + lasalocid 125 mg/kg, iv) Cylactin® at 0.3 x 10⁹ CFU/kg + maduramycin 5 mg/kg, v) Cylactin® at 0.3 x 10⁹ CFU/kg + narasin/nicarbazin 100 mg/kg, vi) Cylactin® at 0.3 x 10⁹ CFU/kg + narasin 70 mg/kg, vii) Cylactin® at 0.3 x 10⁹ CFU/kg + salinomycin 70 mg/kg. Each dietary treatment was assigned to five replicate groups of two birds.

At the end of the study, the microbiota in the ileal chyme and excreta were analysed: the total number of enterococci were enumerated by culture in faeces, quantitative PCR on DNA extracted from ileal chyme was used to enumerate total enterococci, *E. faecium* and the *E. faecium* NCIMB 10415 strain.

The cultivation approach adopted for faecal samples did not allow specific enumeration of *E. faecium* NCIMB 10415. Moreover, the quantitative PCR on DNA extracted from intestinal samples does not allow the differentiation between viable and dead cells. Therefore, the FEEDAP Panel cannot...
conclude on the compatibility of *E. faecium* NCIMB 10415 with lasalocid, maduramycin, narasin/nicarbazin, narasin and salinomycin.

2.6. **Proposed conditions of use**

The product is intended for use in feed for chickens for fattening at a minimum content of $0.3 \times 10^9$ and a maximum content of $2.8 \times 10^9$ CFU/kg of complete feedingstuffs.

2.7. **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 *E. faecium* in animal feed. The Executive Summary of the CRL report can be found in the Appendix.

3. **Safety**

3.1. **Safety for chickens for fattening**

A tolerance study was conducted to evaluate the safety of Cylactin® LBC ME10 in chickens for fattening. The test included 1632 Ross 308 one-day-old chickens randomly distributed by sex into 24 pens and fed one of three dietary treatments: T-1) a negative control, T-2) control + maximum recommended dose Cylactin® (2.8 $\times 10^9$ CFU/kg), T-3) control + 100 times the maximum recommended dose of Cylactin® (2.8 $\times 10^{11}$ CFU/kg). All the doses were confirmed by analyses. The chickens received a starter diet containing 1 mg diclazuril till 21 days of age, and a grower diet containing 100 mg/kg monensin till the end of the experiment at 35 days. Diets were pelleted at a temperature below 65 °C. Performance was calculated for each period and for the overall experiment. There were four replicates of males and four replicates of females per treatment.

When used at 100 times the recommended dose, Cylactin® did not show any adverse effect on performance (body weight: T1 2.12 kg, T2 2.17 kg, T3 2.21 kg) or mortality (T1 2.9 %, T2 1.5 %, T3 2.6 %).

3.2. **Safety for the consumer**

*Enterococcus faecium* NCIMB 10415 is free of known enterococcal virulence factors and does not harbour acquired genes coding for antibiotic resistance.

The metabolism of *E. faecium* is well known and when the virulence factors are excluded, and no other harmful metabolites or substances are expected to be produced during fermentation. In addition, cells are washed prior to incorporation to the additive and therefore any carry-over from the fermentation would be negligible. Consequently, the FEEDAP Panel does not see the need for toxicological studies. This view is supported by the history of use, both in animals and directly in humans.

3.3. **Safety for the user**

Cylactin® LBC ME20 plus did not cause skin irritation in any of the three rabbits tested following a four-hour semi-occlusive skin exposure performed according to OECD Guideline 404.

Eye irritancy of Cylactin® LBC ME20 plus was tested in rabbits according to OECD Guideline 405. No adverse effects were observed in two out of three rabbits. In the third rabbit, at one hour after treatment, there was slight discharge, mild reddening of the conjunctiva and of the sclera, and mild swelling of the conjunctiva. However, at 24 hours post-treatment and at later observations, no adverse effects were seen. It can be concluded that the test material caused transient irritation in the eyes of rabbits and should be regarded as being non-irritant to mucosal membranes.

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35 Technical dossier/Section IV/Appendix 4-2.
36 Technical dossier/Supplementary information Dec 09/Appendix 4-10.
37 Technical dossier/Supplementary information Dec 09/Appendix 4-11.
The skin sensitisation potential of Cylactin® LBC ME20 plus has been studied in a mouse local lymph node assay, involving 13 mice, which gave a negative result. The study was performed in accordance with OECD Guideline 429.38

As the product is essentially dust-free, inhalation exposure of users will be minimal.15 16

3.4. Safety for the environment

Enterococcus faecium is a natural component of the gut microbiota of animals and the use of E. faecium NCIMB 10415 as Cylactin® in animal feeding would not be expected to cause a significant increase of the species in the environment.

4. Efficacy for chickens for fattening

Three trials with Cylactin® LBC ME10 at different doses, including the minimum recommended dose of 0.3 x 10^9 CFU/kg of complete feedingstuffs, were provided by the applicant. An additional large scale trial was performed to support efficacy. Two of these trials were conducted with diets containing lasalocid sodium or salinomycin sodium, coccidiostats for which compatibility with Cylactin® has not yet been demonstrated. In all the experiments, intended Cylactin® supplementation was confirmed by analysis. Two other experiments were not considered due to the high doses used (higher than ten-fold overdose).

In the first trial, 1600 Ross 308 one-day-old male birds were distributed to two experimental groups (control, Cylactin® at 0.3 x 10^9 CFU/kg feed) and grown up to 42 days.39 Each group was composed of 20 replicates and 40 birds per replicate. The animals were fed a typical pelleted cereal-soybean diet. Overall, mortality was low (3 %) and not influenced by treatment. Weight gain and feed to gain ratio were significantly improved in treated animals (Table 2).

A second trial was performed over two consecutive growth cycles (runs I and II), each from day one to day 36, in the same room, with an intermediate period of three weeks.40 For each run, 400 Ross PM3 one-day-old male birds were distributed to two experimental groups (control, Cylactin® at 0.35 x 10^9 CFU/kg feed –analysed values were found to be between 0.51 x 10^9 CFU/kg feed (run I) and 1.25 x 10^9 CFU/kg feed (run II)). Each group was composed by eight replicates and 25 birds per replicate. The animals were fed a typical cereal-soybean diet containing the coccidiostat lasalocid sodium. Overall, mortality was between 6.0 and 7.5 % and not influenced by treatment. In both runs, weight gain was significantly improved in treated animals (Table 2).

In a third trial, 2112 Ross PM3 one-day-old sexed birds were distributed to four experimental groups (control, Cylactin® at 0.3 x 10^9 CFU/kg feed, Cylactin® at 1 x 10^9 CFU/kg feed and Cylactin® at 10 x 10^9 CFU/kg feed) and grown up to 42 days.41 Each group was composed of 12 replicates of 44 birds each (22 males and 22 females). The animals were fed a typical cereal-soybean diet (presence of a diclazuril in the starter phase and no coccidiostat in the finisher phase). Overall, mortality was low (1.3–3.2 %) and not related to treatment. Weight gain was significantly improved in treated animals at the dose of 0.3 x 10^9 CFU/kg feed (Table 2).

In a large scale experiment, 5824 Ross 308 one-day-old birds (both sexes) were distributed to two experimental groups (control, Cylactin® at 0.3 x 10^9 CFU/kg mash feed) and grown up to 49 days.42 Cylactin® content in the supplemented diet was found to be 1 x 10^9 CFU/kg feed. Each group was composed of 16 replicates and 182 birds per replicate. The animals were fed a typical cereal-soybean diet in mash form. Diclazuril was included in the starter diet and salinomycin in the grower diet. Feed consumption and body weight (20% of animals of each replicate) were recorded. Overall, mortality

38 Technical dossier/Supplementary information Dec 09/Appendix 4-9.
39 Technical dossier/Section III/Appendix 3-7.
40 Technical dossier/Section III/Appendix 3-8.
41 Technical dossier/Section III/Appendix 3-9.
42 Technical dossier/Section III/Appendix 3-10.
was found between 7.0 % and 7.7 % and not related to treatment. Weight gain and feed to gain ratio were significantly improved in treated animals at the dose of 0.3 x 10^9 CFU/kg feed (Table 2).

**Table 2:** Summary of the efficacy trials with Cylactin® in chickens for fattening

<table>
<thead>
<tr>
<th>Trial</th>
<th>Duration (days)</th>
<th>Dose (CFU/kg)</th>
<th>Final weight (kg)</th>
<th>Feed intake (kg)</th>
<th>Feed/gain (kg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>0 0.3 x 10^9</td>
<td>2.39^a 2.50^b</td>
<td>4.95 4.98</td>
<td>2.07^a 1.99^b</td>
</tr>
<tr>
<td>2 (run 1)</td>
<td>36</td>
<td>0 0.5 x 10^9</td>
<td>2.08^a 2.25^b</td>
<td>3.44^a 3.72^b</td>
<td>1.65 1.66</td>
</tr>
<tr>
<td>(run 2)</td>
<td>36</td>
<td>0 1.25 x 10^9</td>
<td>2.16^b</td>
<td>3.72^b</td>
<td>1.72</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>0 0.3 x 10^9</td>
<td>2.33^a 2.37^b</td>
<td>4.04 4.11</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x 10^9</td>
<td>2.36^ab</td>
<td>4.14</td>
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<td></td>
<td></td>
<td>10 x 10^9</td>
<td>2.35^ab</td>
<td>4.08</td>
<td>1.76</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>0 0.3 x 10^9</td>
<td>1.68^a 1.76^b</td>
<td>3.36 3.43</td>
<td>2.18^a 2.09^b</td>
</tr>
</tbody>
</table>

1 In trials 1 and 2 values are total weight gain, in trials 3 and 4 values are final body weight.

a,b: Different superscripts within the row indicate levels of significance at P < 0.05.

5. **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 and Good Manufacturing Practice.

**CONCLUSIONS**

When used at 100 times the recommended dose, Cylactin® did not show any adverse effect on the performance or mortality of chickens for fattening. Therefore, the additive is safe for the target animals at the proposed conditions of use.

*Enterococcus faecium* NCIMB 10415 is free of known virulence factors. Resistance to kanamycin shown in this strain is most likely to be caused by an unknown mechanism that potentiates the effect of the sfkmr gene, and not by acquisition of genes coding for aminoglycosides-modifying enzymes; and is thus not a cause for concern. No other concerns for consumer safety were identified.

Cylactin® is not a skin/mucosal irritant or skin sensitiser. As the product is essentially dust-free, inhalatory exposure of users will be minimal.

Cylactin® is efficacious in chickens for fattening as demonstrated in four trials showing an increase in final body weight when diets were supplemented at or close to the minimum recommended dose (0.3 x 10^9 CFU/kg feed).

Cylactin® is compatible with the coccidiostats decoquinate, monensin, robenidine, diclazuril and semduramycin.

**DOCUMENTATION PROVIDED TO EFSA**


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5. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Cylactin®.

6. Comments from Member States received through the ScienceNet.

**REFERENCES**


EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on additives and products or substances used in animal feed (FFEDAP) on the updating of the criteria used in the assessment of the bacteria for resistance to antibiotics of human or veterinary importance. The EFSA Journal (2005) 223, 1-12.


EFSA (European Food Safety Authority), 2008b. 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. The EFSA Journal 658, 1-5.

APPENDIX

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

In the current application authorisation is sought for the microbial feed additive Cylactin/Cernivet LBC ME 10 and 20 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 NCIMB 10415. The additive is available in micro-encapsulated form and contains a minimum concentration of $1 \times 10^{10}$ colony forming units (c.f.u.) per gram. Specifically, authorisation is sought to use Cylactin/Cernivet LBC ME 10 and 20 for chickens for fattening. The conditions of use are proposed with a recommended dosage of $0.3$ to $2.8 \times 10^9$ c.f.u./kg feed.

For the quantification of the active agent (Enterococcus faecium NCIMB 10415) of Cylactin/Cernivet LBC ME 10 and 20 in the feed additive, premixtures and feedingstuffs, an appropriate spread plate method was proposed by the applicant. The method was in-house validated and the method precision data 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 fully ring-trial validated spread plate enumeration method is recommended (J. Appl. Microbiol. 2002, 93, 781-786).

The method’s performance characteristics of the enumeration method are standard deviations for repeatability ($s_r$) and reproducibility ($s_R$) of around $0.12 - 0.20 \log_{10}$ and $0.23 - 0.41 \log_{10}$ calculated from the base 10 logarithms of the measured c.f.u./g in feedingstuffs, respectively. The limits of quantification (LOQ) of this method are around $10^4$ colony forming units (c.f.u.) per gram (g) feed additive or premixture and around $10^7$ c.f.u./kg feedingstuff.

The identity of the bacterial strain, Enterococcus faecium NCIMB 10415, was analysed by microscopy, biochemistry and molecular methods such as randomly amplified polymorphic DNA (RAPD) methodology. Pulsed-field gel electrophoresis (PFGE) is recognised as a 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.