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Safety and efficacy of *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529) as a feed additive for chickens for fattening

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of *Lactobacillus acidophilus* D2/CSL when used in feed for chickens for fattening at a minimum dose of 1×10^9 colony-forming units (CFU)/kg complete feedingstuffs. The additive is a preparation of viable cells of *L. acidophilus*. This species is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establish safety for the target species, consumers and the environment. The safety of *Lactobacillus acidophilus* CECT 4529 was assessed by EFSA in 2014. Following the QPS approach to safety assessment, *Lactobacillus acidophilus* CECT 4529 is assumed to be safe for the target species, the consumer and the environment without the need for further studies. No concerns are expected from other excipients present in the product, so *Lactobacillus acidophilus* D2/CSL is also considered safe for target animals, including chickens for fattening, consumers and the environment. The safety of the additive for the user was also considered in that opinion. The FEEDAP Panel is unaware of any new data that would lead it to revise its conclusions that the additive should be considered to be an eye/skin irritant and a skin/respiratory sensitiser. There is insufficient evidence to conclude on the efficacy of the additive when used in diets for chickens for fattening.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 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 Centro Sperimentale del Latte S.r.l.¹ for authorisation of the product *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529), when used as a feed additive for chickens for fattening (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). EFSA received directly from the applicant the technical dossiers in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 21 September 2015.

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 safety for the target animals, consumer, user and the environment and on the efficacy of the product *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529), when used under the proposed conditions of use (see Section 3).

1.2. Additional information

The additive is a preparation containing viable cells of *Lactobacillus acidophilus* CECT 4529. EFSA issued an opinion on the safety and efficacy of this product when used with laying hens (EFSA FEEDAP Panel, 2014).

The additive is currently authorised for use in laying hens.²

The species *Lactobacillus acidophilus* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to establishing safety for the target species, consumers and the environment (EFSA, 2007; EFSA BIOHAZ Panel, 2013).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier³ in support of the authorisation request for the use of *Lactobacillus acidophilus* D2/CSL as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁴ and the applicable EFSA guidance documents.

The European Union Reference Laboratory considered that the conclusions and recommendations reached in the previous assessment are valid and applicable for the current application.⁵

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lactobacillus acidophilus* D2/CSL 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, 2012a), Technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012b).

¹ Centro Sperimentale del Latte S.r.l., strada del Merlino 3, 26839, Zelo Buon Persico, Italy.

² Commission Implementing Regulation (EU) 2015/38 of 13 January 2015 concerning the authorisation of the preparation of *Lactobacillus acidophilus* CECT 4529 as a feed additive for laying hens and amending Regulation (EC) No 1520/2007 (holder of authorisation Centro Sperimentale del Latte). OJ L 8, 14.1.2015, p. 4.

³ FEED dossier reference: FAD-2015-0022.

⁴ Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

⁵ The full report is available on the EURL website: <https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0394.pdf>

3. Assessment

The additive is a preparation of viable cells of a single strain of *L. acidophilus* intended for use as a zootechnical additive (gut flora stabiliser) in feed for chickens for fattening. It has a minimum guaranteed concentration of 5×10^{10} CFU of *Lactobacillus acidophilus* CECT 4529 per gram of additive.

The additive under assessment has the same formulation and method of manufacture as that considered in the previous opinion (EFSA FEEDAP Panel, 2014). Thus, the data pertaining to composition, impurities, physical properties and shelf life still apply. The data on stability in premixtures, mash feed and pelleted feed for laying hens are considered applicable to chickens for fattening, given the likely similarity in feed formulation.

Lactobacillus acidophilus D2/CSL is intended for use in feed for chickens for fattening at a minimum dose of 1×10^9 CFU/kg complete feedingstuffs.

3.1. Safety

In its opinion on the use of *Lactobacillus acidophilus* D2/CSL in feed for laying hens, the FEEDAP Panel concluded that *Lactobacillus acidophilus* CECT 4529 meets the requirements for the QPS safety assessment, and therefore can be presumed safe for target species, consumers of products derived from animals fed the additive and the environment (EFSA FEEDAP Panel, 2014). These conclusions are considered still to be valid. No concerns are expected from other excipients present in the product, so *Lactobacillus acidophilus* D2/CSL is also considered safe for target animals, including chickens for fattening, consumers and the environment.

In the context of the same opinion, it was concluded that the additive should be considered to be an eye/skin irritant and a skin/respiratory sensitiser. The use of the additive in diets for chickens for fattening is considered unlikely to introduce hazards for users of the product not already considered.

3.2. Efficacy

A total of six studies conducted in the same Member State but in three different locations were submitted. However, one study⁶ was not further considered due to flaws in the experiment (e.g. lack of replication, conditions of use different from the proposed ones⁷), and two⁸ other due to unusually high mortality and low growing performance of birds. In each of the remaining cases, trials consisted of a comparison of a control group with a group given the additive at the minimum recommended dose. The total numbers of birds used and number of replicates per treatment for each trial are shown in Table 1. The birds involved were male 1-day-old Ross 308 in trials 1 and 2 and Kabir birds in trial 3. The applicant justifies the choice of this slow growing breed with the intention to simulate conditions present in small farming systems.

In the trials considered, the additive was administered via the feed during the whole experimental period (41 days in trial 1,⁹ 45 days in trial 2¹⁰ and 42 days in trial 3¹¹) at the minimum recommended dose of 1×10^9 CFU/kg feed. Concentration in feed was confirmed by analysis. In all cases, starter and grower diets based on maize/soybean meal/wheat in mash form were provided *ad libitum*. Live weights of birds and feed intake were recorded over time and weight gain and feed to gain calculated. Morbidity and mortality were monitored. In all trials, performance data were subjected to analysis of variance (ANOVA) and differences were compared with Students' t-test. The pen was the experimental unit for all parameters. Mortality data were analysed using non-parametric tests in study 3.

In trial 2, data from all time-point measurements were considered by the applicant and the model included treatment and time as main factors, with a significant outcome for feed intake. When the statistical analysis of feed intake in this study was repeated by the Working Group considering only the total intake at 45 days, differences among groups were found not to be significant. Birds given feed supplemented with the additive showed significantly improved feed to gain ratio in two out of the three studies and significantly improved weight gain/final weight in one of these. No other parameter was significantly affected by treatment. Therefore, based on the data provided (only two studies with

⁶ Technical dossier/Section IV/Annex IV.28.

⁷ Additive given via water for drinking at the dose of 1×10^8 CFU/bird at three time intervals.

⁸ Technical dossier/Section IV/Annexes IV.29, 29a, 29b, 31c, 32 and IV.31, 31.a, 31.b, 31.c, 32.

⁹ Technical dossier/Section IV/Annexes IV.30, 30.a, 30.b, 30.c, 30.d, 30.e, 30.f and 32.

¹⁰ Technical dossier/Supplementary information November 2016/A.

¹¹ Technical dossier/Supplementary information November 2016/B.

positive results on the performance of the birds receiving the additive), the FEEDAP Panel cannot conclude on the efficacy of *Lactobacillus acidophilus* D2/CSL in chickens for fattening.

Table 1: Summary of performance data of chickens for fattening receiving *Lactobacillus acidophilus* D2/CSL

Trial	Total animals (replicates/ treatment x animals/ replicate)	<i>Lactobacillus acidophilus</i> D2/CSL (CFU/kg feed)	Feed intake (g/bird/day)	Final weight (kg)	Weight gain (g/bird/day)	Feed:gain (g/g)	Mortality (%) ⁽¹⁾
1	800 (16 × 25)	0 1.0 × 10 ⁹	106.2	2.76	65.8	1.61 ^(b)	2
			105.3	2.78	66.3	1.59 ^(a)	3
2	276 (6 × 23)	0 1.0 × 10 ⁹	104.9	2.99	n.r.	1.58	2
			103.4	2.97		1.57	0
3	264 (6 × 22)	0 1.0 × 10 ⁹	86.3	1.44 ^(a)	35.0 ^(a)	2.94 ^(b)	10
			85.4	1.64 ^(b)	39.8 ^(b)	2.56 ^(a)	8

CFU: colony-forming unit; n.r.: Not reported.

(a),(b): Means in a column within a trial with a different superscript letters are significantly different ($p < 0.05$).

(1): Not statistically analysed in study 2.

3.3. 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 Regulation¹² and Good Manufacturing Practice.

4. Conclusions

Lactobacillus acidophilus CECT 4529 fulfils the requirements of the QPS approach to the assessment of safety and no concerns are expected from other components of the additive. Consequently, *Lactobacillus acidophilus* D2/CSL can be presumed safe for chickens for fattening, consumers of products derived from animals fed the additive and environment.

The additive *Lactobacillus acidophilus* D2/CSL should be considered to be an eye/skin irritant and a skin/respiratory sensitiser.

There is insufficient evidence to conclude on the efficacy of the additive when used in diets for chickens for fattening.

Documentation provided to EFSA

- 1) Dossier of the zootechnical additive *Lactobacillus acidophilus* D2/CSL (CECT 4529) (4b1715) concerning a new use of the feed additive for the animal category chickens for fattening according to article 4 (1) of Regulation (EC) No 1831/2003. July 2015. Submitted by CSL Centro Sperimentale del Latte S.p.A.
- 2) *Lactobacillus acidophilus* D2/CSL (CECT 4529) (4b1715) concerning a new use of the feed additive for the animal category chickens for fattening according to article 4 (1) of Regulation (EC) No 1831/2003. Supplementary information. November 2011. Submitted by CSL Centro Sperimentale del Latte S.p.A.
- 3) *Lactobacillus acidophilus* D2/CSL (CECT 4529) (4b1715) concerning a new use of the feed additive for the animal category chickens for fattening according to article 4 (1) of Regulation (EC) No 1831/2003. Supplementary information. November 2016. Submitted by CSL Centro Sperimentale del Latte S.p.A.
- 4) Comments from Member States.

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¹² Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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Abbreviations

ANOVA	analysis of variance
CFU	colony-forming unit
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
QPS	qualified presumption of safety