Safety and efficacy of the feed additive consisting of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus D2/CSL*) for all poultry species and categories and all ornamental birds (Centro Sperimentale del Latte S.r.l)

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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 the feed additive consisting of *Lactobacillus acidophilus* CECT 4529 (tradename: *Lactobacillus acidophilus D2/CSL*) when used as a zootechnical additive for all poultry species and categories and all ornamental birds. The additive is authorised for use in laying hens, chickens for fattening, cats and dogs. With this application, the company requested a new authorisation for use in feed and water for drinking for all poultry species and categories and ornamental birds. The Panel concluded that *Lactobacillus acidophilus D2/CSL* is presumed safe for the target species, consumers and the environment. As in previous opinions, the Panel also concluded that it is considered an eye/skin irritant and a skin/respiratory sensitiser. *Lactobacillus acidophilus D2/CSL* when supplemented at $1 \times 10^9$ CFU/kg complete feed or $5 \times 10^8$ CFU/L water for drinking has the potential to be efficacious in to improving either laying or growing performance in all poultry species/categories and ornamental birds.

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**Keywords:** Zootechnical additive, gut flora stabiliser, *Lactobacillus acidophilus CECT 4529*, *Lactobacillus acidophilus D2/CSL*, chickens for fattening, safety, efficacy

**Requestor:** European Commission

**Question number:** EFSA-Q-2020-00847

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA’s work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgments: The Panel wishes to thank the following for the support provided to this scientific output: FEEDAP Working Group on Microbiology, FEEDAP Working Group on Animal Nutrition, Elisa Pettenati and Martina Reitano.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Bampidis V, Azimonti G, Bastos ML, Christensen H, Dusemund B, Fasmon Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F, Mayo B, Pechova A, Petkova M, Ramos F, Sanz Y, Villa RE, Woutersen R, Galobart J, Vittoria Vettori M and Brozzi R, 2022. Scientific Opinion on the safety and efficacy of the feed additive consisting of Lactobacillus acidophilus CECT 4529 (Lactobacillus acidophilus D2/CSL) for all poultry species and categories and all ornamental birds (Centro Sperimentale del Latte S.r.l). EFSA Journal 2022;20(3):7150, 9 pp. https://doi.org/10.2903/j.efsa.2022.7150

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.
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1. **Introduction**

1.1. **Background and Terms of Reference**

Regulation (EC) No 1831/2003\(^1\) 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\(^2\) for the authorisation of the additive consisting of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL), when used as a feed additive for all poultry species and categories and ornamental birds (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 dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 9 March 2021.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL), when used under the proposed conditions of use (see Section 3.1.3).

1.2. **Additional information**

EFSA has issued three opinions on the safety and efficacy of this product when used in feed for laying hens (EFSA FEEDAP Panel, 2014), chickens for fattening (EFSA FEEDAP Panel, 2017a) and cats and dogs (EFSA FEEDAP Panel, 2018a).

The additive is currently authorised for use in feed for laying hens,\(^3\) chickens for fattening\(^4\) and cats and dogs\(^5\) (4b1715).

2. **Data and methodologies**

2.1. **Data**

The present assessment is based on data submitted by the applicant in the form of a technical dossier\(^6\) in support of the authorisation request for the use of the product consisting of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL) as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts’ knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active agent in animal feed are valid and applicable for the current application.\(^7\)

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\(^1\) Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

\(^2\) Centro Sperimentale del Latte S.r.l, Strada per Merlino 3, 26839, Zelo Buon Persico, Italy.

\(^3\) 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 authorization Centro Sperimentale del Latte).

\(^4\) Commission Implementing Regulation (EU) 2017/2275 of 8 December 2017 concerning the authorisation of a new use of the preparation of *Lactobacillus acidophilus* CECT 4529 as a feed additive for chickens for fattening (holder of the authorisation Centro Sperimentale del Latte).

\(^5\) Commission Implementing Regulation (EU) 2018/1558 of 17 October 2018 concerning the authorisation of a new use of the preparation of *Lactobacillus acidophilus* CECT 4529 as a feed additive for cats and dogs (holder of the authorisation Centro Sperimentale del Latte). OJ L 261, 18.10.2018, p. 13.

\(^6\) FEED dossier reference: FAD-2020-0009.

\(^7\) The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0394.pdf](https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0394.pdf)
2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of *Lactobacillus acidophilus* CECT 4529 (*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 studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017d), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018b), Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018c) and Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The product consisting of viable cells of *L. acidophilus* CECT 4529 (tradename *Lactobacillus acidophilus* D2/CSL) is intended for use as a zootechnical additive (functional group: gut flora stabilisers) in feed for all poultry species and categories and ornamental birds.

3.1. Characterisation

3.1.1. Characterisation of the active agent

The active agent was isolated from the gastrointestinal tract of chickens and is deposited at the Colección Española de Cultivos Tipo with the accession number CECT 4529. It has not been genetically modified.

The taxonomical identification of the strain CECT 4529 as *L. acidophilus* was confirmed by bioinformatic analysis of the whole genome sequence (WGS) of the active agent. This was based on digital DNA–DNA hybridisation (dDDH) using the DSMZ type strain genome server (TYGS) database. Results showed a dDDH value of 99.8 % compared with the type strain *L. acidophilus* NBRC 13951.7

The strain was tested for antibiotic susceptibility by the broth dilution method. The battery of antibiotics used included those recommended by the FEEDAP Guidance (EFSA FEEDAP Panel, 2018c). The minimum inhibitory concentration values for *L. acidophilus* CECT 4529 fell below or were equal to the EFSA cut-off values, except for ampicillin, which was exceeded by a single dilution. Exceeding the cut-off value by one dilution is considered to be within the normal range of variation, and thus, not a matter of concern. Therefore, the strain is considered to be susceptible to all the relevant antibiotics.

The WGS data of the strain were interrogated for the presence of antimicrobial resistance (AMR) genes using the CARD (perfect, strict and loose hits settings) and ResFinder (thresholds: 80% for identity and 60% for minimum length) databases. No hits were identified in CARD using the Perfect and Strict RGI criteria (all loose hits < 58% identity) or in ResFinder applying the afore-mentioned thresholds.

3.1.2. Characterisation of the additive

The additive under assessment has the same formulation (10–30% bacterial cells and 70–90% lactose/maltodextrin) and method of manufacture as that considered in the previous opinions (EFSA FEEDAP Panel, 2014, 2017a, 2018a). It ensures a minimum guaranteed concentration of $5 \times 10^{10}$.
colony forming units (CFU) of *L. acidophilus* CECT 4529 per gram of additive. The data pertaining to composition, physical properties and stability submitted in the previous application dossiers still apply. However, this application contains new data that are described below.

Analytical data to confirm the specifications were provided for ten batches of the additive, showing an average value of $6.5 \times 10^{10}$ CFU/g and a range of $3.3 - 12 \times 10^{10}$ CFU/g.\(^{14}\)

Microbiological contamination was analysed on the same ten batches showing compliance with the established specifications: Enterobacteriaceae ($< 100$ CFU/g), yeasts and filamentous fungi ($< 100$ CFU/g), *Escherichia coli* (not detected in 1 g), *Salmonella* spp. (not detected in 25 g), *Staphylococcus aureus* (not detected in 25 g) and *Listeria monocytogenes* (not detected in 25 g).\(^{14}\)

Four batches of the additive were analysed for chemical impurities.\(^{15}\) Heavy metals (cadmium, lead and mercury), arsenic and aflatoxin M1 levels were below the limit of detection (LOD) of the analytical methods, except for one batch showing 0.0144 mg As/kg.\(^{16}\)

The detected amounts of the above-described impurities do not raise safety concerns.

### 3.1.3. Conditions of use

*Lactobacillus acidophilus* D2/CSL is intended for use in feed and water for drinking for all poultry species and categories (birds reared for fattening, growing birds, pullets, layers, breeders), and for all ornamental birds at a concentration of $1 \times 10^9$ CFU/kg of complete feed or $5 \times 10^8$ CFU/L water for drinking.

### 3.2. Safety

#### 3.2.1. Safety for the target species, consumers and environment

The bacterial species *L. acidophilus* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach (EFSA, 2007; EFSA BIOHAZ Panel, 2021). This approach requires the identity of the strain to be conclusively established and evidence that it does not harbour acquired antimicrobial resistance genes. In the view of the FEEDAP Panel, the identity of the active agent as *L. acidophilus* was established, and the compliance with the absence of acquired antimicrobial resistance genes was confirmed. Therefore, the Panel concludes that *L. acidophilus* CECT 4529 can be presumed safe for target animals, consumers and the environment. Since no concerns are expected from other excipients present in the product, the additive *Lactobacillus acidophilus* D2/CSL is also considered safe for target animals, consumers and the environment.

#### 3.2.2. Safety for the user

In the context of a previous opinion (EFSA FEEDAP Panel, 2014), the additive was considered an eye/skin irritant and a skin/respiratory sensitiser. No new information supporting the safety of the additive for the user has been submitted in the current application. The use of the additive in diets for the new target species is considered unlikely to introduce hazards for users of the product not already considered. Therefore, the conclusions reached in the previous assessment apply to the current application.

### 3.3. Efficacy

In 2014, the FEEDAP Panel concluded, based on the results from 3 studies in laying hens, that the ‘administration of *Lactobacillus acidophilus* D2/CSL at the proposed level ($1 \times 10^9$ CFU/kg feed) significantly increased laying intensity and improved the feed-to-egg mass ratio in all three trials. Egg weight was also significantly increased in two of the three trials.’ Therefore, the additive was considered to have the potential to be efficacious in laying hens at $1 \times 10^9$ CFU/kg feed or at the equivalent concentration in water of $5 \times 10^8$ CFU/L.

In 2017, the Panel assessed the efficacy of the additive in chickens for fattening and concluded that positive and significant effects on feed to gain ratio were found in only two studies. Therefore, the Panel could not conclude on the efficacy of the additive in chickens for fattening.

The applicant in the current application has submitted a new efficacy study in chickens for fattening which is described below.

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\(^{14}\) Technical dossier/Section II/Annexes II_2, II_2.1, II_2.2, II_2.3, II_2.4, II_2.5.

\(^{15}\) Technical dossier/Section II/Annexes II_10, II_11, II_12, II_12a.

\(^{16}\) LOD: Cd: 0.005 mg/kg, Hg: 0.005 mg/kg, Pb: 0.005 mg/kg, aflatoxin M1: 0.05 µg/kg.
3.3.1. Efficacy for chickens for fattening

A total of 276 one-day-old male chickens for fattening (Ross 308) were distributed in 12 pens in groups of 23 animals and allocated to two dietary treatments (six replicates per treatment). Three basal diets (starter, grower 1 and grower 2) based on maize, wheat, and soya bean meal, were either not supplemented (control) or supplemented with Lactobacillus acidophilus D2/CSL to provide $1 \times 10^9$ CFU per kg feed (confirmed by analysis). Diets were offered on ad libitum basis in mash form for 42 days. Mortality and health status were checked every day and dead animals were necropsied. Animals were weighed on days 1, 7, 14, 21, 28, 35 and 42 (individual basis), feed intake was registered per pen and feed to gain ratio calculated. The data were statistically analysed considering treatment, time (repeated measurements) and their interaction as the effects, and the pen (within treatment) as the experimental unit. Mortality was analysed using the Fisher test. Significance level was set at 0.05. The results are presented in Table 1.

Overall mortality was low (2.1%) and not affected by treatment. Birds receiving L. acidophilus D2/CSL showed a reduced feed to gain ratio and greater body weight gain compared to those in the control group, with no interaction with time. The body weight and the total feed intake showed interactions between the treatment and the time, indicating improvements in the birds receiving L. acidophilus D2/CSL from day 21 and 35, respectively.

3.3.1.1. Conclusions on efficacy for chickens for fattening

Based on the results reported in this study and those previously evaluated by the FEEDAP Panel (EFSA FEEDAP Panel, 2017a), the Panel concludes that the additive has the potential to be efficacious in chickens for fattening at the recommended inclusion level of $1 \times 10^9$ CFU/kg of complete feed.

The concentration proposed for use in water for drinking ($5 \times 10^8$ CFU/L) would provide essentially the same daily dose as that proposed in complete feed. Consequently, the conclusions on efficacy of the additive when used in feedingstuffs also apply to the use in water for drinking for chickens for fattening.

3.3.2. Efficacy for all poultry species and categories and ornamental birds

In the current application, the applicant is seeking for an extension of use of the additive in all poultry species and categories and in ornamental birds at $1 \times 10^9$ CFU/kg complete feed and $5 \times 10^8$ CFU/L water.

The efficacy of Lactobacillus acidophilus D2/CSL at the same inclusion level was established for chickens for fattening in the studies described above and in laying hens in a previous opinion (EFSA FEEDAP Panel, 2014). The FEEDAP Panel considers that the conclusions reached in laying hens and chickens for fattening can be extended/extrapolated to all poultry species/categories and ornamental birds when used at the same use level.

3.4. 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.

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Table 1: Effects of Lactobacillus acidophilus D2/CSL on the performance of chickens for fattening

| Trial | Lactobacillus acidophilus D2/CSL (CFU/kg feed) | Total feed intake (g) | Body weight (g) | Mean weekly body weight gain (g) | Feed to gain ratio | Mortality (%) |
|-------|-----------------------------------------------|-----------------------|----------------|---------------------------------|-------------------|--------------|
|       |                                               | Initial               | Final          |                                 |                   |              |
| 1     | 0                                             | 5,134<sup>a</sup>     | 42.8           | 2,979<sup>b</sup>             | 1.72<sup>a</sup>  | 2.45         |
|       | $1 \times 10^9$                               | 5,034<sup>b</sup>     | 42.8           | 3,016<sup>a</sup>             | 1.67<sup>b</sup>  | 1.73         |

CFU: colony forming unit.

<sup>a,b</sup>: Mean values within a column with a different superscript are significantly different ($p < 0.05$).

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17 Technical dossier/Section IV/Annex 8.a-n and Supplementary information September_2021/EFSA_Answers_D2_CSL_15.09.2021, Original Study Protocol D2_CSL, SAS_output_A_BW_15.09.2021 and SAS_output_B_BW_15.09.2021.

18 Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.
4. Conclusions

*Lactobacillus acidophilus* D2/CSL is safe for the target species, consumers and the environment. *Lactobacillus acidophilus* D2/CSL is an eye/skin irritant and a skin/respiratory sensitisier. *Lactobacillus acidophilus* D2/CSL when supplemented at $1 \times 10^9$ CFU/kg complete feed or $5 \times 10^8$ CFU/L water for drinking has the potential to be efficacious in improving either laying or growing performance in all poultry species/categories and ornamental birds.

5. Documentation provided to EFSA/Chronology

| Event                                                                 | Date       |
|----------------------------------------------------------------------|------------|
| Dossier received by EFSA. Application for authorisation of *Lactobacillus acidophilus* D2/CSL-CECT 4529 (EU No 4b1715) for a new use in feed and water for drinking for all poultry species and categories and ornamental birds. Submitted by Centro Sperimentale del Latte s.r.l. | 03/12/2020 |
| Reception mandate from the European Commission                        | 23/12/2020 |
| Application validated by EFSA – Start of the scientific assessment    | 09/03/2021 |
| Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: characterisation | 20/05/2021 |
| Reception of supplementary information from the applicant - Scientific assessment re-started | 09/07/2021 |
| Requests of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. Issues: efficacy | 10/06/2021 |
| Request of supplementary information from the applicant - Scientific assessment re-started | 05/08/2021 |
| Reception of supplementary information from the applicant - Scientific assessment re-started | 17/09/2021 |
| Opinion adopted by the FEEDAP Panel. End of the Scientific assessment | 26/01/2022 |

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Abbreviations

AMR antimicrobial resistance
CECT Colección Española de Cultivos Tipo
CFU colony forming unit
dDDH digital DNA–DNA hybridisation
DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen
EURL European Union Reference Laboratory
FEEDAP EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD limit of detection
QPS qualified presumption of safety
TYGS type strain genome server
WGS whole genome sequence