

Assessment on Safety and Efficacy of a 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) (RP1742)

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FSA Research and Evidence

An application was submitted to the Food Standards Agency in September 2022 from Centro Sperimentale del Latte S.r.l. ("the applicant") for the new use (extension of species) of an additive consisting of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL), under the category of 'zootechnical additive' and functional group 'gut flora stabilisers' for its use in all poultry and all ornamental birds.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) concluded that *Lactobacillus acidophilus* D2/CSL is safe for the consumer, target species and environment. The Panel concluded that the additive should be considered an eye and skin irritant and a respiratory and skin sensitiser. The additive has the potential to be efficacious in all poultry species and ornamental birds when supplemented at 1×10^9 CFU/kg complete feed or 5×10^8 CFU/L drinking water.

The FSA/FSS has reviewed the application, supporting documentation, and other regulators risk assessments, most notably the EFSA risk assessment opinion, and considers that sufficient evidence has been demonstrated to conclude without the need for further questions or risk assessment.

This is a joint FSA and FSS publication.



1. Introduction

The FSA and FSS have undertaken an assessment of a feed additive (Centro Sperimentale del Latte S.r.l., Strada per Merlino, 3, 26839, Zelo Buon Persico (Lodi), Italy) consisting of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL) under Assimilated Regulation (EC) No 1831/2003 (EC, 2003) in each nation of Great Britain (GB) for a new use (extension of species) under category of 'zootechnical additive' and functional group 'gut flora stabilisers' for its use in all poultry species and ornamental birds. Under Assimilated Regulation (EU) No 2015/38, 2017/2275 and 2018/1558 (EC, 2015, 2017, 2018), the additive is currently authorised for use in feed for laying hens, chickens for fattening, and cats and dogs.

In line with Article 8 of Assimilated Regulation (EC) No 1831/2003, the assessment has considered whether the feed additive complies with the conditions laid down in Article 5, including: safety considerations for human, animal and environmental health; efficacy of the additive for its intended effect; potential impairment of the distinctive features of animal products. This, and the guidance put in place by the European Food Safety Authority (EFSA) for the evaluation of feed additive applications, has formed the basis and structure for the assessment.

To ensure the regulatory systems of FSA/FSS are risk proportionate and resources are used effectively, FSA and FSS have used the evidence submitted by the applicant and other information in the public domain, including the EFSA risk assessment opinion, to provide a summary assessment of the evidence of safety presented in this report.

In 2022, EFSA published a risk assessment opinion (EFSA, 2022) on the new use of *Lactobacillus acidophilus* CECT 4529 as a feed additive for all poultry species and ornamental birds (Centro Sperimentale del Latte S.r.l.). This opinion has been reviewed by the FSA/FSS. It has been verified that the standard approach taken, when compared to the relevant guidance applied in GB, has been followed and the conclusions made are consistent with the data summarised in the opinion.

The result of the assessment is that there is sufficient evidence of safety for the UK to conclude this assessment at this time. This assessment represents the opinion of the FSA and FSS.

Table 1. Table showing products included in this assessment

Title	Product type	Intended use/s	Intended dose/intake
<i>Lactobacillus acidophilus</i> CECT 4529	Feed additive	Zootechnical	• 1×10^9 CFU/kg complete feed or; • 5×10^8 CFU/L water for drinking

2. Assessment

2.1. Details of other regulators opinions

2.1.1. Previous authorisations and opinions

The EFSA FEEDAP Panel previously issued three opinions on the safety and efficacy of *Lactobacillus acidophilus* CECT 4529 (EFSA, 2014, 2017d, 2018c), which is currently authorised for use in laying hens, chickens for fattening, and cats and dogs. For all three assessments the FEEDAP Panel concluded that *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL) can be presumed safe for the target species, the consumer and the environment, with *Lactobacillus acidophilus* considered suitable for the qualified presumption of safety (QPS) approach to assessment, owing to the identity of the strain being established and the absence of antimicrobial resistance genes demonstrated. The Panel concluded that the additive was correctly characterised and that the additive is a skin and eye irritant, and a skin and respiratory sensitiser.

The additive was concluded to be efficacious for laying hens at the proposed inclusion rate, and the potential to be efficacious was concluded for use in cats and dogs. A conclusion on efficacy could not be drawn in chickens for fattening. As a part of the new use application, the applicant has provided an updated efficacy study for chickens for fattening.

2.1.2. Methodology applied in the EFSA opinion

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) assessed the safety and the efficacy of *Lactobacillus acidophilus* CECT 4529 (*Lactobacillus acidophilus* D2/CSL), in accordance with the following guidance documents:

- Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a);

- 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, 2017b);
- Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018a);
- Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018b);
- Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019);
- Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012);

and principles in Assimilated Regulation (EC) No 429/2008 (EC, 2008).

These guidance documents were developed and implemented prior to the UK's exit from the EU and were adopted by the FSA and FSS on exit.

2.2. Section II: Identity, characterisation and condition of use

The additive is composed of viable cells of *Lactobacillus acidophilus* CECT 4529, originally isolated from the gastrointestinal tract of chickens, that have not been genetically modified. Taxonomic identification was achieved through bioinformatic interrogation of whole genome sequencing of the active agent. Antimicrobial susceptibility was investigated through broth dilution, with all minimum inhibitory concentrations below the EFSA cut-offs, except for ampicillin (a single dilution greater than the cut-off). The FEEDAP Panel concluded that this was within the normal range of variation and not a cause for concern. The presence of AMR genes was investigated, and no hits were reported.

The additive's manufacturing process and specifications are the same as previously assessed by EFSA (2014, 2017d, 2018c). The FEEDAP Panel determined that the information previously assessed for composition, physical properties and stability still apply and the extrapolation to new species did not raise any safety concerns. The FEEDAP Panel concluded that the active agent was fully characterised (EFSA, 2022).

The FSA and FSS agree with the conclusions reached for the characterisation of the additive and active agent. The studies were reviewed by EFSA in 2014, 2017 and 2018, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. These studies formed part of the

assessment leading to the current authorisation of the additive in GB. The certificates of analysis were reviewed by the FSA and FSS and confirmed the compliance with the specifications. The identity and the manufacturing process of the additive is not changed for the current application made to the FSA and FSS, and as such has not been subject to further assessment. The characterisation of the feed additive is provided as per the existing authorisation and as assessed by EFSA.

2.2.2. Condition of use

Lactobacillus acidophilus D2/CSL is intended for use in all poultry species and categories, and for all ornamental birds at a concentration of 1×10^9 CFU/kg of complete feed or 5×10^8 CFU/L water for drinking.

The applicant requested the same conditions of use as EFSA evaluated in their latest opinion (EFSA, 2022). The FSA/FSS agree with the conditions of use proposed by the applicant.

2.2.3. Conclusion on Section II

The additive was fully characterised, and the identity of the active agents confirmed in the previous EFSA opinions (EFSA, 2014, 2017d, 2018c). The Panel reviewed the updated information provided for assessment, confirming taxonomical identification of the active agents and demonstrating susceptibility to the relevant antibiotics (EFSA, 2022).

The FSA/FSS agree with the conclusions reached on the data, which is supported by the guidance that is also applicable in GB.

2.3. Section III: Safety

2.3.1. Safety for the target animals, consumers and the environment

In its previous opinions, the Panel concluded that the active agent met the requirements for the QPS approach to assessment and were presumed safe for the target animal, consumers and the environment (EFSA, 2014, 2017a and 2018a). The applicant did not provide any new data to make the FEEDAP Panel reconsider previous conclusions. The Panel concluded that the proposed new use would not influence these previous conclusions.

The FSA and FSS agree with the conclusions reached on the data and the use of the QPS approach, as this approach has been previously used in GB and decisions on QPS were made while the UK were part of the EU.

2.3.2. Safety for the user

In a previous opinion (EFSA, 2014), the Panel concluded that the additive was an eye and skin irritant, and a skin and respiratory sensitiser, owing to the absence of data and considering the proteinaceous nature of the additive. The applicant did not provide any new data to make the FEEDAP Panel reconsider this conclusion when used in all poultry species and all ornamental birds.

The FSA and FSS agree with the conclusions reached for the safety of the user. The information was originally assessed by EFSA in 2014, prior to the UK's exit from the EU; thus, this opinion is applicable to GB. These studies formed part of the assessment leading to the current authorisation of the additive in GB.

2.3.3. Conclusion on Section III: Safety

The FEEDAP Panel concluded that the new use of the additive would not affect the previous conclusions for safety for the target species, consumer or the environment. The additive should be considered an eye and skin irritant, and a skin and respiratory sensitiser.

The FSA/FSS agree with the conclusions reached on the data, which is supported by the guidance that is also applicable in GB.

2.4. Efficacy

In 2014 the FEEDAP panel concluded that the additive had the potential to be efficacious in laying hens at 1×10^9 CFU/kg or at 5×10^8 CFU/L (EFSA, 2014). In 2017, the FEEDAP panel were unable to conclude on the efficacy of the additive in chickens for fattening as positive effects on feed to gain ratio were only noted in two of the efficacy trials (EFSA, 2017a). In 2018, the FEEDAP Panel concluded that the additive had the potential to be efficacious in cats and dogs, however, the biological relevance of the observed effect was questionable (EFSA, 2018a). For the current authorisation, the applicant provided a new efficacy study in chickens for fattening to support the new use of the additive in all poultry species and ornamental birds.

2.4.1. Efficacy for chickens for fattening

EFSA reviewed the new efficacy study in their latest opinion (EFSA, 2022). A study was presented with 276 one-day-old male Ross 308 chickens for fattening divided into 12 pens (23 animals/pen); the effects of the study are summarised in [Table 2](#).

Table 2. Effects of *Lactobacillus acidophilus* D2/CSL on the performance of chickens for fattening

Trial	Lactobacillus acidophilus D2/CSL CECT 4529 (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 ^a	42.8	2,979 ^b	489 ^b	1.72 ^a	2.45
	1 x 10 ⁹	5,034 ^b	42.8	3,016 ^a	496 ^a	1.67 ^b	1.73

a, b: Mean values within a column with a different superscript are significantly different (P < 0.05).

Birds receiving *Lactobacillus acidophilus* D2/CSL showed a reduced feed to weight gain ratio and an increased body weight when compared to the control group. Combined with the previous data assessed for chickens for fattening, the Panel concluded that the additive has the potential to be efficacious in chickens for fattening at the proposed inclusion rate.

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

Efficacy for *Lactobacillus acidophilus* D2/CSL was demonstrated at the same inclusion level for chickens for fattening and for laying hens (EFSA, 2014). The EFSA FEEDAP Panel concluded that the conclusions in these species allow the extrapolation to all poultry species and categories and ornamental birds when used at the same concentrations.

2.4.3. Conclusions on Section IV: Efficacy

The FEEDAP Panel concluded that the updated efficacy study in chickens for fattening demonstrated the additive to have the potential to be efficacious at the proposed inclusion rate. The FEEDAP Panel determined that the results of the studies in chickens for fattening combined with the previous data provided for laying hens (EFSA, 2014) could be extrapolated to all poultry species and categories and ornamental birds, concluding that *Lactobacillus acidophilus* D2/CSL has the potential to be efficacious at an inclusion rate of 1 x 10⁹ CFU/kg complete feed or 5 x 10⁸ CFU/L drinking water.

The FSA/FSS agree with the conclusions reached on the data and the extrapolation to all poultry species and categories and ornamental birds, which is supported by the guidance that is also applicable in GB. This demonstrates that *Lactobacillus acidophilus* D2/CSL favourably affects animal production, performance or welfare, particularly by affecting the gastrointestinal flora of the target species.

3. Analytical methods evaluation

The FSA/FSS evaluated the EURL analytical method evaluation, noting it was carried out in 2010, when the UK was still part of the EU and would have participated in their approval. No concerns are raised at this stage for the validity of the methods for UK/GB use, and therefore, the FSA/FSS accept the EURL analytical method evaluation report (EURL, 2010). FSA/FSS determined the analytical method as appropriate for official controls for this feed additive.

4. Conclusions

The FEEDAP Panel concluded that *Lactobacillus acidophilus* D2/CSL can be considered safe for the target species, consumer and the environment. The additive is considered a skin and eye irritant and a skin and respiratory sensitiser. The additive has the potential to be efficacious for all poultry species and all ornamental birds at an inclusion rate of 1×10^9 CFU/kg complete feed or 5×10^8 CFU/L drinking water.

5. Caveats and uncertainties

No caveats or uncertainties have been identified.

6. FSA/FSS conclusions for GB risk analysis

The application has been assessed in line with the applicable guidance and is partially based on considerations of detailed proprietary information available to the Panel, which were also submitted to the FSA and FSS. The EFSA opinion identifies and characterises the hazards present from the new use of the feed additive consisting of *Lactobacillus acidophilus* D2/CSL, and there is sufficient information to enable an assessment of exposure, which is also relevant to GB. The risk characterisation is unchanged from the 2014, 2017 and 2018 opinions, and appropriate evidence was submitted to support the requested new use. The conclusions of the EFSA opinion have been reviewed in detail by the FSA and FSS and are considered appropriate and consistent, including the caveats and uncertainties identified in the opinion which are applicable to GB. Sufficient evidence has been demonstrated to conclude without further questions or risk assessment.

Abbreviations

Abbreviation	Definition
EC	European Commission
CFU	Colony forming units
GB	Great Britain

Abbreviation	Definition
FSA	Food Standards Agency
FSS	Food Standards Scotland
EFSA	European Food Safety Authority
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
EURL	European Union Reference Laboratory
QPS	Qualified presumption of safety

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References

- EC (European Commission). (2003). *Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition*. <https://www.legislation.gov.uk/eur/2003/1831/contents>
- EC (European Commission). (2008). *Regulation No 429/2008 of the European Parliament and of the Council on additives for use in animal nutrition*. <https://www.legislation.gov.uk/eur/2008/429/contents>
- EC (European Commission). (2015). *Regulation No 2015/38 of the European Parliament and of the Council on additives for use in animal nutrition*. <https://www.legislation.gov.uk/eur/2015/38>
- EC (European Commission). (2017). *Regulation No 2017/2275 of the European Parliament and of the Council on additives for use in animal nutrition*. <https://www.legislation.gov.uk/eur/2017/2275>
- EC (European Commission). (2018). *Regulation No 2018/1558 of the European Parliament and of the Council on additives for use in animal nutrition*. <https://www.legislation.gov.uk/eur/2018/1558>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2012). Guidance on studies concerning the safety of use of the additive for users/workers. *EFSA Journal*, 10(1), 2539. <https://doi.org/10.2903/j.efsa.2012.2539>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2014). Scientific Opinion on the safety and efficacy of *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529) as a feed additive for laying hens. *EFSA Journal*, 12(7), 3789. <https://doi.org/10.2903/j.efsa.2014.3789>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017a). Guidance on the assessment of the safety of feed additives for the consumer. *EFSA Journal*, 15(10), 5022. <https://doi.org/10.2903/j.efsa.2017.5022>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017b). Guidance on the assessment of the safety of feed additives for the target species. *EFSA Journal*, 15(10), 5021. <https://doi.org/10.2903/j.efsa.2017.5021>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017c). Guidance on the identity, characterisation and conditions of use of feed additives. *EFSA Journal*, 15(10), 5023. <https://doi.org/10.2903/j.efsa.2017.5023>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2017d). Scientific Opinion on the safety and efficacy of *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529) as a feed additive for chickens for fattening. *EFSA Journal*, 15(4), 4762. <https://doi.org/10.2903/j.efsa.2017.4762>
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018a). Guidance on the assessment of the efficacy of feed additives. *EFSA Journal*, 16(5), 5274. <https://doi.org/10.2903/j.efsa.2018.5274>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018b). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, 16(3), 5206. <https://doi.org/10.2903/j.efsa.2018.5206>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2018c). Scientific Opinion on the safety and efficacy of *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529) as a feed additive for cats and dogs. *EFSA Journal*, 16(5), 5278. <https://doi.org/10.2903/j.efsa.2018.5278>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2019). Guidance on the assessment of the safety of feed additives for the environment. *EFSA Journal*, 17(4), 5648. <https://doi.org/10.2903/j.efsa.2019.5648>

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2022). Scientific Opinion on the safety and efficacy of *Lactobacillus acidophilus* D2/CSL (*Lactobacillus acidophilus* CECT 4529) as a feed additive for all poultry species and categories and all ornamental birds. *EFSA Journal*, 20(3), 7150. <https://doi.org/10.2903/j.efsa.2022.7150>

EURL-FA (European Reference Laboratory for Feed Additives). (2010). *Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation of a Feed Additive according to Regulation (EC) No 1831/2003. Lactobacillus acidophilus D2/CSL*. <https://joint-research-centre.ec.europa.eu/system/files/2013-02/FinRep-FAD-2010-0394.pdf>