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

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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 cats and dogs at a minimum dose of 5×10^9 colony forming units (CFU)/kg complete feedingstuffs. The additive is a preparation of viable cells of *Lactobacillus acidophilus* CECT 4529. This species is considered by the European Food Safety Authority to be suitable for the qualified presumption of safety (QPS) approach to establishing safety for the target species and the environment. The safety of *L. acidophilus* CECT 4529 was assessed by EFSA in 2014. Following the QPS approach to safety assessment, *L. acidophilus* CECT 4529 is assumed to be safe for the target species 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 cats and dogs, 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. *Lactobacillus acidophilus* D2/CSL has some potential to reduce the moisture of stools from dogs and cats receiving the additive at 5 x 10^9 CFU/kg feed. However, the biological relevance of the magnitude of the effect detected is questionable.

Keywords: zootechnical additive, gut flora stabilisers, *Lactobacillus acidophilus* D2/CSL, safety, efficacy, dogs, cats

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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 dogs and cats (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 9 August 2017.

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, 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 3.1.1).

1.2. Additional information

The additive *Lactobacillus acidophilus* D2/CSL is a preparation containing viable cells of *Lactobacillus acidophilus* CECT 4529. EFSA issued an opinion on the safety and efficacy of this additive when used with laying hens (EFSA FEEDAP Panel, 2014) and chickens for fattening (EFSA FEEDAP Panel, 2017).

The additive is currently authorised as a zootechnical additive (functional group: gut flora stabilisers) for use in laying hens[2] and chickens for fattening.[3]

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 and the environment (EFSA, 2007; EFSA BIOHAZ Panel, 2017).

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[4] 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 429/2008[5] 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.[6]

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, 2012),

[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] 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. OJ L 326, 9.12.2017, p. 47

[4] FEED dossier reference: FAD-2017-0035.

[5] 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.

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

Technical guidance on tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a) and Guidance on the assessment of additives intended to be used in pets and other non food-producing animals (EFSA FEEDAP Panel, 2011b).

3. Assessment

The additive is a preparation of *L. acidophilus* CECT 4529 intended for use as a zootechnical additive (gut flora stabiliser) in feed for cats and dogs.

3.1. Characterisation of the additive

Lactobacillus acidophilus D2/CSL has a minimum guaranteed concentration of 5 x 10^{10} CFUs of *L. acidophilus* CECT 4529 per gram of additive. It was fully characterised in previous opinions (EFSA FEEDAP Panel, 2014; EFSA FEEDAP Panel, 2017). It has the same formulation (bacterial cells and lactose) and method of manufacture as that considered in the previous opinions, thus, the data pertaining to composition, impurities, physical properties and shelf life still apply.

A study was performed to investigate the stability of three batches of the additive when incorporated in dry feed for cats and dry feed for dogs, and stored at 4°C and 25°C, for 2, 4, 8 and 13 weeks.[7] At 4°C, losses in counts were negligible up to 8 weeks but were > 0.5 log at 13 weeks in both cases, whilst reached losses ≥ 0.5 log after 4 weeks at 25°C in both cases. A second study involved extruded kibbles for dogs (produced by sprinkling the extruded feed with a premixture of hydrolysed proteins and the additive at 35°C) in sealed aluminium-polythene bags and stored at 4°C or 25°C for 2, 4, 8 and 13 weeks. Results were similar to those obtained with the feeds used in the first study.

The capacity of the additive to homogeneously mix with pet food was tested using 15 sub-samples of the three feeds mentioned above.[7] Viable counts of *L. acidophilus* CECT 4529 showed a coefficient of variation of 6% for the dry feeds and of 7% for the extruded feed, denoting good capability of the additive to homogeneously mix with pet food.

3.1.1. Conditions of use

Lactobacillus acidophilus D2/CSL is intended for use in feed for dogs and cats at a minimum dose of 5 × 10^9 CFU/kg complete feedingstuffs. The applicant declares that the feed additive can be added to premixtures and complete feeds provided that they are not pelleted above 52°C.

3.2. Safety

In its opinion on the use of *Lactobacillus acidophilus* D2/CSL in feed for laying hens, the FEEDAP Panel concluded that *L. acidophilus* CECT 4529 meets the requirements for the QPS safety assessment, and therefore can be presumed safe for target species 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 the additive *Lactobacillus acidophilus* D2/CSL is also considered safe for target animals (cats and dogs) 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 cats and dogs 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

The additive is intended to stabilise the microbiota in the gastrointestinal tract of dogs and cats to improve stool consistency. In line with the current guidance on additives intended to be used in pets, three studies with dogs and one with cats were submitted to support the efficacy of the additive.

3.4. Efficacy for dogs

The studies were conducted with healthy adult dogs of both sexes and of different breeds and body weight (Table 1), fed a commercial dry extruded dog food. The experimental design was the

[7] Technical dossier/Section II/Annex 22a.

same in the three studies, comprising two experimental groups with different numbers of observations (Table 1). In each study, dogs were randomly allocated to the two dietary treatments: control and *Lactobacillus acidophilus* D2/CSL.

Table 1: Summary of the experimental design of trials testing efficacy the *Lactobacillus acidophilus* D2/CSL in dogs

Trial	*Lactobacillus acidophilus* D2/CSL (CFU/kg feed)	No of animals (sex) per group Breed Body weight (kg)	Measurements (time points, days)
1[(a)]	0 5×10^9	6 (3♂, 3♀) 9 (4♂, 5♀) English Cocker Spaniel 12–13 kg	Body weight and body condition score (0, 14, 28 and 35) Faecal moisture and score (0, 14, 28 and 35)
2[(b)]	0 5×10^9	16 (2♂, 14♀) 24 (3♂, 21♀) Boxer 23–24 kg	Body weight and body condition score (0, 7, 14, 21, 28 and 35) Faecal moisture, hardness and score (0, 14, 21, 28 and 35)
3[(c)]	0 5×10^9	6 (2♂, 4♀) 9 (3♂, 6♀) Labrador 30–31 kg	Body weight and body condition score (0, 14, 28 and 35) Faecal moisture and score (0, 14, 28 and 35)

CFU: colony forming unit.
(a): Technical dossier/Section IV/Annexes IV_28.
(b): Technical dossier/Section IV/Annexes IV_29.
(c): Technical dossier/Section IV/Annexes IV_30.

After a 7-day period of acclimatisation, dogs received the experimental diets for 35 days. Dogs were housed in kennels with two or three animals per cage. Dogs in the same kennel received the same experimental diet. The feed for the animals in the *L. acidophilus* group included the additive incorporated via a premixture (with maltodextrins) to deliver a minimum concentration of 5×10^9 CFU of lactobacilli per kg feed. Compliance of the additive with the specifications and the nominal concentration of the additive in the treated feed were confirmed by analysis. Animals in the control group received the same feed mixed with the same amount of maltodextrins but without additive. The daily amount of feed given to the dogs was established from their breed and metabolic weight ($bw^{0.75}$) and recorded. Within each kennel, feed was dispensed in individual bowls (one identifiable dish for each dog), and the consumption of all the amount of food by each animal was strictly controlled by a kennel operator to prevent bowl change and/or competitive/aggressive behaviour. Individual body weight and body condition score (BCS) were monitored at different time points (Table 1). Faecal samples were collected on site immediately upon defecation from each dog at the same measurement days, and used to determine moisture content of stools and faecal score using a 7-point-scale (with 1 denoting very hard and dry faeces and 7 watery faeces). Stool hardness using a penetrometer was also measured in study 2. Data on body weight and faecal moisture were subjected to analysis of variance (ANOVA), with sampling times as repeated measurements. The individual animal was used as the experimental unit. Body condition scores and faecal scores were compared by the non-parametric Kruskall–Wallis test. Statistical significance was declared at p-value < 0.10.

Table 2: Effects of *Lactobacillus acidophilus* D2/CSL on faecal consistency in dogs

Study	*Lactobacillus acidophilus* D2/CSL (CFU/kg food)	Faecal moisture (g/g faeces) on day in study					Faecal score[(a)] on day			
		0	14	28	35	Overall	0	14	28	35
1	0	0.65	0.60	0.71	0.67	0.66	3.0	2.7	3.8	3.0
	5×10^9	0.65	0.60	0.62*	0.66	0.63*	3.0	2.2	2.6*	3.2
2	0	0.66	0.66	0.73	0.69	0.69	4.9	3.9	3.9	3.9
	5×10^9	0.68	0.63	0.68*	0.68	0.67*	4.8	3.1	3.3*	3.3*

Study	*Lactobacillus acidophilus* D2/CSL (CFU/kg food)	Faecal moisture (g/g faeces) on day in study					Faecal score[(a)] on day			
		0	14	28	35	Overall	0	14	28	35
3	0	0.68	0.64	0.72	0.67	0.68	4.3	3.7	3.2	3.2
	5×10^9	0.65	0.59*	0.68*	0.66	0.64*	3.9	2.7*	3.1	3.1

CU: colony forming unit.
*: Control and additive groups significantly different at $p < 0.10$.
(a): 1 denoting very hard and dry faeces and 7 watery faeces.

There were no differences between treatments in the dog body weight at any of the sampling times. No effects of the treatment were observed on body condition score. In the three studies, faecal moisture at day 28 was significantly reduced in dogs receiving the additive in comparison with the control group but this effect was not observed at day 35 (Table 2). However, the analysis of the overall data showed a small but significant reduction in faecal moisture in all cases.

In study 2, faeces hardness was increased with the additive in stools collected at days 21 (control: 0.88 kg, additive: 1.11 kg, $p = 0.002$) and 35 (control: 0.82 kg, additive: 1.09 kg, $p = 0.0002$). Faecal score was reduced (more consistent stools) with the additive at days 28 (studies 1 and 2) and 35 (only study 2). In study 3, faecal score was significantly reduced with the additive only at day 14 (3.7 vs. 2.7 for control and additive groups, respectively). An overall analysis of faecal scores is not considered appropriate.

The results of faecal moisture were variable with respect to time and showed no significant effects at the end of the studies. However, overall analysis of the data showed a small but significant reduction in faecal moisture in all three studies. The biological relevance of the differences detected is questionable.

3.5. Efficacy for cats

A trial following the same design described above but with ten adult Maine Coon cats (7 females and 3 males of 3–9 kg body weight) was conducted.[8] Cats (1 male and 4 females in the control group and 2 males and 3 females in the additive group) receiving the same dietary treatment were housed in the same room. Cats were fed a commercial dry extruded food with or without *Lactobacillus acidophilus* D2/CS. The additive was incorporated into the feed via a premixture (with maltodextrins) to deliver a minimum concentration of 5×10^9 CFU of lactobacilli per kg. Compliance of the additive with the specifications and of the concentration of the additive in the treated feed was confirmed by analysis. In each room, food was dispensed in individual bowls (one identifiable dish for each cat), and the consumption of all the amount of food by each animal was strictly controlled by a qualified carer to prevent bowl change and/or competitive/aggressive behaviour and recorded. The individual animal was considered the experimental unit (5 per treatment). Body weight and BCS were monitored on days 0, 7, 14, 21, 28 and 35. Faecal samples (stools taken on site immediately upon defecation from each cat) were collected at the same time points to determine moisture content and faecal score using the same aforementioned scale. Body weight and faecal moisture data were subjected to ANOVA using sampling times as repeated measurements. BCS and faecal scores were compared by using the non-parametric Kruskall–Wallis test. Statistical significance was declared at p-value < 0.10.

Table 3: Effects of *Lactobacillus acidophilus* D2/CSL on faecal consistency in cats

Lactobacillus acidophilus D2/CSL (CFU/kg food)	Faecal moisture (g/g faeces) on day					Faecal score[(a)] on day			
	0	14	28	35	Overall	0	14	28	35
0	0.47	0.43	0.46	0.47	0.46	3.8	3.8	4.0	3.4
5×10^9	0.45	0.42	0.44	0.43*	0.44*	4.0	3.2	3.2*	3.0

CFU: colony forming unit.
*: Control and additive groups significantly different at $p < 0.10$.
(a): 1 denoting very hard and dry faeces and 7 watery faeces.

[8] Technical dossier/Section IV/Annex IV_31.

There were no differences between treatments in the cat body weight at any of the sampling times. The results of faecal moisture were variable with respect of time and were not affected by treatment at any time except on day 35 (Table 3). However, the overall analysis of the data showed a significant reduction in faecal moisture. As with dogs, the biological relevance of the differences detected is questionable.

3.6. Conclusions on efficacy for dogs and cats

The FEEDAP Panel concludes that supplementation of *Lactobacillus acidophilus* D2/CSL has some potential to reduce the moisture of stools from dogs and cats receiving the additive at 5×10^9 CFU/kg feed. However, the biological relevance of the magnitude of the effect detected is questionable.

3.7. 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[9] 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, the additive *Lactobacillus acidophilus* D2/CSL can be presumed safe for cats and dogs and the environment.

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

Lactobacillus acidophilus D2/CSL has some potential to reduce the moisture of stools from dogs and cats receiving the additive at 5×10^9 CFU/kg feed. However, the biological relevance of the magnitude of the effect detected is questionable.

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 dogs and cats 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 dogs and cats according to article 4 (1) of Regulation (EC) No 1831/2003. Supplementary information. December 2017. Submitted by CSL Centro Sperimentale del Latte S.p.A.
3) Comments from Member States.

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[9] 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.

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Abbreviations

ANOVA	analysis of variance
BCS	body condition score
bw	body weight
CFU	colony forming unit
QPS	Qualified presumption of safety