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## **Safety and efficacy of a feed additive consisting of *Lactiplantibacillus plantarum* DSM 11520 for horses, dogs, cats and pet rabbits (Animal Probiotics Sweden AB)**

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### **Abstract**

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on *Lactiplantibacillus plantarum* DSM 11520 when used as a technological additive (acidity regulator) in feed for horses, dogs, cats and pet rabbits. The additive is intended to be incorporated into oat-derived products (ca. 55% moisture content), carrot root-derived products ( $\geq 90\%$  moisture) and coconut flesh-derived products ( $\geq 90\%$  moisture) at a minimum inclusion level of  $8.0 \times 10^{10}$  CFU/kg of the feed material under scope. The bacterial species *L. plantarum* is considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment. The identity of the strain has been clearly established and it did not show acquired resistance to antibiotics of human and veterinary importance. The FEEDAP Panel concluded that the use of this strain in animal nutrition is safe for the target species, consumers of horse meat and the environment. Regarding the user safety, the additive *Lactiplantibacillus plantarum* DSM 11520 is not irritant to skin or eyes in the product tested containing maltodextrin and oat bran as carriers, but owing to its proteinaceous nature, it should be considered a respiratory sensitiser. No conclusions can be drawn on the skin sensitisation potential of the additive. The FEEDAP Panel concluded that incorporation of *Lactiplantibacillus plantarum* DSM 11520 at a minimum concentration of  $8.0 \times 10^{10}$  CFU/kg into oat-derived products (ca. 55% moisture content), carrot root-derived products ( $\geq 90\%$  moisture) and coconut flesh-derived products ( $\geq 90\%$  moisture) has the potential to reduce the pH of these feedingstuffs.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Animal Probiotics Sweden AB<sup>2</sup> for the authorisation of the additive consisting of *Lactiplantibacillus plantarum* DSM 11520 when used as a feed additive for horses, dogs, cats and pet rabbits (not food-producing rabbits) (category: technological; functional group: acidity regulator).

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 21 January 2022.

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 *Lactiplantibacillus plantarum* DSM 11520, when used under the proposed conditions of use (see **Section 3.1.4**).

### 1.2. Additional information

The additive is a preparation containing *Lactiplantibacillus plantarum* DSM 11520 (formerly known as *Lactobacillus plantarum*). It is not currently authorised in the European Union. The FEEDAP Panel delivered an opinion on the same active agent when used as a zootechnical additive (EFSA FEEDAP Panel, 2020).

## 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<sup>3</sup> in support of the authorisation request for the use of *Lactiplantibacillus plantarum* DSM 11520 as a feed additive. The dossier was received on 19/11/2021 and the general information and supporting documentation is available at <https://open.efsa.europa.eu/questions/EFSA-Q-2021-00687>.

In accordance with Article 38 of the Regulation (EC) No 178/2002<sup>4</sup> and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,<sup>5</sup> a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 11 July to 1 August 2022 for which no comments were received.

In addition, the confidential version of the technical dossier was subject to a target consultation of the interested Member States from 14 March to 14 June 2022 for which received comments that were considered for the assessment.

<sup>1</sup> 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.

<sup>2</sup> Animal Probiotics Sweden AB, Ideon, SE-223 70 – Lund, Sweden.

<sup>3</sup> FEED dossier reference: FEED-2021-2210.

<sup>4</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

<sup>5</sup> Decision available online: <https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements>

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the active agent in animal feed.<sup>6</sup>

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of the active agent is in line with the principles laid down in Regulation (EC) No 429/2008<sup>7</sup> 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, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), 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) and EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021).

## 3. Assessment

The product under assessment is based on a preparation of viable cells of a single strain of *L. plantarum* (formerly known as *Lactobacillus plantarum*) intended to be used as a technological additive (functional groups: acidity regulator) in feed for horses, dogs, cats and pet rabbits.

### 3.1. Characterisation

#### 3.1.1. Characterisation of the active agent

The strain was originally isolated from a healthy horse.<sup>8</sup> It is deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) under the accession number DSM 11520.<sup>9</sup> It harbours two plasmids and has not been genetically modified.

The strain DSM 11520 was identified at species level as *L. plantarum* by bioinformatic analysis of the whole genome sequence (WGS) data.<sup>10</sup> Average nucleotide identity (ANI) gave a value of 99.27% compared to the genome sequence of the type strain *L. plantarum* ATCC 14917<sup>T</sup>.

The susceptibility of the strain was tested using a broth microdilution method against the list of antimicrobials recommended by EFSA (EFSA FEEDAP Panel, 2018a). All the minimum inhibitory concentration (MIC) values determined were equal to or fell below the corresponding cut-off values defined by the FEEDAP Panel, except for kanamycin, which showed a MIC of 128 mg/L, which is one dilution above the cut-off.<sup>11</sup> Exceeding the cut-off by one dilution is considered to be within the normal range variation of the technique and thus, not a matter of concern. Therefore, the strain DSM 11520 is considered to be susceptible to all relevant antibiotics.

The WGS of the strain, including plasmids, was interrogated for the presence of antimicrobial resistance (AMR) genes against the [REDACTED] and [REDACTED] databases. A threshold of 70% identity and 60% length coverage was set at both nucleotide and protein levels.<sup>10</sup> No hits of concern were identified.

#### 3.1.2. Characterisation of the additive

The active agent is grown [REDACTED]. The final product (hereby referred to as 'additive') is in a powder form and it is standardised by mixing the freeze-dried cell concentrate [REDACTED] with oat bran [REDACTED] and maltodextrin [REDACTED] as carriers,

<sup>6</sup> The full report is available on the EURL website: [https://joint-research-centre.ec.europa.eu/publications/feed-2021-2210\\_en](https://joint-research-centre.ec.europa.eu/publications/feed-2021-2210_en)

<sup>7</sup> 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.

<sup>8</sup> Identity\_characterisation.

<sup>9</sup> Annex\_II\_2\_1\_2\_1.

<sup>10</sup> Annex\_II\_2\_1\_2\_2.

<sup>11</sup> Annex\_II\_2\_2\_2\_1.

to reach a minimum guaranteed concentration of  $1.3 \times 10^{10}$  colony forming units (CFU) per gram of additive. No antimicrobials are used during the manufacturing process.<sup>12</sup>

Analytical data to confirm the specifications were provided for five batches of the additive showing an average of  $2.4 \times 10^{10}$  CFU/g additive, ranging between  $2.1$  and  $2.6 \times 10^{10}$  CFU/g.<sup>13</sup>

Analyses of three batches of the additive confirmed compliance with the specifications set by the applicant for yeasts, filamentous fungi and Enterobacteriaceae ( $< 10^2$  CFU/g), coagulase-positive staphylococci ( $< 10^2$  CFU/g), *Bacillus cereus* ( $< 10^3$  CFU/g) and *Salmonella* spp. and *Listeria monocytogenes* (both no detection in 25 g).<sup>14</sup>

Three batches of the additive were analysed for the presence of cadmium, lead, mercury, arsenic, aflatoxins (B1, B2, G1 and G2), ochratoxin A, deoxynivalenol, zearalenone, fumonisin B1 and B2 and for HT2 and T2 toxins.<sup>15</sup> Values were below the limit of quantification (LOQ) of the corresponding analytical methods.<sup>16</sup>

The FEEDAP Panel considers that the microbial contamination and the amounts of the detected impurities do not raise safety concerns.

A set of physico-chemical parameters were determined in three batches of the additive.<sup>17</sup> The average bulk density obtained was  $397 \text{ kg/m}^3$  (range:  $379$ – $425 \text{ kg/m}^3$ ). The solid density averaged  $1,391 \text{ kg/m}^3$  (range:  $1,380$ – $1,398 \text{ kg/m}^3$ ). The dusting potential measured with the Stauber–Heubach dust meter indicated that the product is dust-free. The particle size distribution of the same three batches was determined using laser diffraction. Results showed that on average 24.91% (v/v) of the additive consist of particles with diameter below  $100 \mu\text{m}$ , 12.59% below  $50 \mu\text{m}$  and 4.70% below  $10 \mu\text{m}$ .

### 3.1.3. Stability and homogeneity

Shelf-life was assessed in four batches of the additive stored in the original packaging (laminated aluminium foil sticks with barriers to moisture and gases) at  $22^\circ\text{C}$  and  $30^\circ\text{C}$  for 15 months (one replicate per batch at each experimental condition). Losses of the active agent counts were  $< 0.5$  Log in all cases.<sup>18</sup>

A short-term stability study was conducted to monitor viability of *L. plantarum* DSM 11520 when the additive is mixed with three different feed materials (crushed oats with added water (ratio 1:1), carrot juice or coconut drink) at a target concentration of  $8.0 \times 10^{10}$  CFU/kg. The samples (one batch of the additive, three replicates per feed material) were incubated following the specified conditions of use in aerobiosis at  $25^\circ\text{C}$  for 24 h. There was no loss but increases of *L. plantarum* after 24 h storage.<sup>19</sup>

### 3.1.4. Conditions of use

The additive is intended to be incorporated into oat-derived products (ca. 55% moisture content), carrot root-derived products ( $\geq 90\%$  moisture) and coconut flesh-derived products ( $\geq 90\%$  moisture) for horses, dogs, cats and pet rabbits at a minimum recommended inclusion level of  $8.0 \times 10^{10}$  CFU/kg of the final feed material under scope. The applicant did not propose a maximum inclusion level in complete feed.<sup>20</sup>

## 3.2. Safety

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

The species *L. plantarum* is considered by EFSA to be eligible for the Qualified Presumption of Safety (QPS) approach (EFSA, 2007; EFSA BIOHAZ Panel, 2023). This approach requires the identity of

<sup>12</sup> Manufacturing process; Annex\_II\_3 and Annex\_II\_1\_3\_1.

<sup>13</sup> Annex\_II\_1\_3\_2.

<sup>14</sup> Annex\_II\_1\_4\_2\_3.

<sup>15</sup> Annex\_II\_1\_4\_2\_4\_chemical\_impurities.

<sup>16</sup> LOQ for cadmium  $0.20 \text{ mg/kg}$ , lead  $2.5 \text{ mg/kg}$ , mercury  $0.02 \text{ mg/kg}$ , arsenic  $2.0 \text{ mg/kg}$ , aflatoxin (B1, B2, G1 and G2) and ochratoxin A  $0.5 \mu\text{g/kg}$ , deoxynivalenol and fumonisin B1 and B2  $40 \mu\text{g/kg}$ , zearalenone  $10 \mu\text{g/kg}$  and for HT2 and T2 toxins  $20 \mu\text{g/kg}$ .

<sup>17</sup> Annex\_II\_1\_5\_1.

<sup>18</sup> Annex\_II\_4\_1\_1.

<sup>19</sup> Annex\_II\_4\_1\_2.

<sup>20</sup> Annex\_II\_5\_1\_Conditions\_of\_use\_RFI-March22.

the strain to be conclusively established and evidence that it does not harbour acquired resistance to antibiotics of human and veterinary importance. In the view of the FEEDAP Panel, the identity of the strain has been established as *L. plantarum* and the antibiotic resistance qualification met. Consequently, *L. plantarum* DSM 11520 is presumed to be safe for the target species, consumers of horse meat and the environment.

### 3.2.2. Safety for the user

No specific studies investigating the effects of the additive on the respiratory system were submitted.<sup>21</sup> The dusting potential data (see Section 3.1.2) indicates that the additive is dust-free, hence, the exposure of the users to dust from the additive can be considered negligible. However, given its proteinaceous nature, the additive is considered a respiratory sensitizer.

The skin<sup>22</sup> and eye<sup>23</sup> irritation potential was tested according to OECD guidelines 439 and 492, respectively, in one batch of the additive (with maltodextrin and oat bran as carriers as described in the manufacturing). The product is not a skin irritant (UN GHS 'No Category') or an eye irritant (UN GHS 'No Category').

Regarding the skin sensitisation potential, no data was made available. However, the FEEDAP Panel notes that the OECD test guidelines available at present are designed to assess the skin sensitisation potential of chemical substances only, and that currently no validated assays for assessing the sensitisation potential of microorganisms are available.<sup>24</sup>

Once an active agent has been authorised as a technological additive, different preparations can be placed on the market with reference to that authorisation. Consequently, not all preparations can be assessed for user safety. The Panel can only conclude on the product tested.

### 3.2.3. Conclusions on safety

The FEEDAP Panel concludes that *Lactiplantibacillus plantarum* DSM 11520 is safe for the target species, consumers of horse meat and the environment under the proposed conditions of use. Regarding user safety, in the product tested containing maltodextrin and oat bran as carriers, *Lactiplantibacillus plantarum* DSM 11520 is not irritant to skin or eyes but, owing to the proteinaceous nature of the active agent, it is considered a respiratory sensitizer. No conclusions can be drawn on the potential of the additive to cause skin sensitisation.

## 3.3. Efficacy

The additive is intended to reduce the pH of oat-derived products (ca. 55% moisture content), carrot root-derived products ( $\geq 90\%$  moisture) or coconut flesh-derived products ( $\geq 90\%$  moisture). To support the efficacy, three *in vitro* studies were performed mimicking real use conditions. All of them showed a common design and investigated the effects of *Lactiplantibacillus plantarum* DSM 11520 in a range of feed matrices (Table 1).<sup>25</sup>

In these studies, the feed matrices were treated either with the additive (target level of *Lactiplantibacillus plantarum* DSM 11520 in the feed material:  $8.0 \times 10^{10}$  CFU/kg) or with equivalent amounts of the additive carriers (maltodextrin and oat bran) as controls. The inclusion level of the active agent was confirmed by analysis in all cases. Feed matrices were incubated under aerobic conditions at 25°C for 24 h (Table 1). Each study considered a control and a treated group with three replicates. The parameters measured on the feed matrices included pH, total titratable acidity (TTA) and lactic acid concentration. The data were analysed using the one-sided Wilcoxon/Kruskal–Wallis non-parametric test followed by chi-square approximation. The replicate sample was the experimental unit. Differences were considered significant at  $p \leq 0.05$ .<sup>26</sup> The details of the experimental design and results are shown in Table 1.

In study 1, water was added to dry feed material (ratio 1:1).

<sup>21</sup> User\_worker\_safety.

<sup>22</sup> Annex\_III\_3\_1\_2\_1\_skin\_irritancy.

<sup>23</sup> Annex\_III\_3\_1\_2.

<sup>24</sup> [https://www.efsa.europa.eu/sites/default/files/2022-07/feedap20220629-30\\_m.pdf](https://www.efsa.europa.eu/sites/default/files/2022-07/feedap20220629-30_m.pdf)

<sup>25</sup> Annex\_IV\_1\_4\_Character\_matrices.

<sup>26</sup> Annex\_IV\_1\_3\_RFI-Oct22\_stats.pdf and Annex\_IV\_1\_4\_EfficacySum\_NP-Stats.pdf.

**Table 1:** Summary of the *in vitro* trials of different feed matrices inoculated with *Lactiplantibacillus plantarum* DSM 11520

Study	Feed Matrix* (% dry matter)	No replicates Aerobic incubation conditions	Treatment <i>Lactiplantibacillus</i> <i>plantarum</i> DSM 11520 (CFU/kg)	pH			Total titratable acidity (TTA) (mL of 0.1 N NaOH/10 g sample)		Lactic acid (g/100 g sample)**	
				0 h	24 h	Δ	0 h	24 h	0 h	24 h
1 <sup>27</sup>	Crushed oats, soaked (ratio matrix:water 1:1) (55.1%)	3 24 h, 25 °C	Control	5.98	5.18 <sup>a</sup>	-0.79 <sup>a</sup>	2.10	6.50 <sup>a</sup>	< 0.05	< 0.05 <sup>a</sup>
			8.0 × 10 <sup>10</sup>	5.98	3.84 <sup>b</sup>	-2.15 <sup>b</sup>	2.10	16.00 <sup>b</sup>	-	1.10 <sup>b</sup>
2 <sup>28</sup>	Carrot juice (6.9%)	3 24 h, 25 °C	Control	6.38	5.49 <sup>a</sup>	-0.88 <sup>a</sup>	0.90	2.00 <sup>a</sup>	< 0.05	0.22 <sup>a</sup>
			8.0 × 10 <sup>10</sup>	6.38	4.21 <sup>b</sup>	-2.18 <sup>b</sup>	0.90	4.23 <sup>b</sup>	-	0.58 <sup>b</sup>
3 <sup>29</sup>	Coconut drink (7.4%)	3 24 h, 25 °C	Control	6.42	5.65 <sup>a</sup>	-0.78 <sup>a</sup>	0.50	0.90 <sup>a</sup>	< 0.05	< 0.05 <sup>a</sup>
			8.0 × 10 <sup>10</sup>	6.42	3.91 <sup>b</sup>	-2.51 <sup>b</sup>	0.50	3.70 <sup>b</sup>	-	0.35 <sup>b</sup>

\*: Total sugar expressed as glucose for matrices in studies 1–3 were 0.36%, 4.77% and 0.64%, respectively.

<sup>a,b</sup>: Values in the same column within study are significantly different with  $p \leq 0.05$ .

\*\*<sup>a</sup>: Limit of detection (LOD) for lactic acid: 0.05 g/100 g sample.

Δ: pH modification from 0 to 24 h.

-: Not measured.

In the three studies, a significantly lower pH was observed in the treated samples compared with controls. Additionally, treated samples had statistically significant greater content of lactic acid compared to the controls, which is in agreement with the statistically significant higher TTA in treated groups compared to controls.

### 3.3.1. Conclusions on efficacy

Based on the three *in vitro* studies, the FEEDAP Panel concludes that *Lactiplantibacillus plantarum* DSM 11520 at a minimum concentration of  $8.0 \times 10^{10}$  CFU/kg has the potential to reduce the pH of oat-derived products (ca. 55% moisture content), carrot root-derived products ( $\geq 90\%$  moisture) and coconut flesh-derived products ( $\geq 90\%$  moisture).

## 4. Conclusions

The additive consisting of *Lactiplantibacillus plantarum* DSM 11520 is safe for the target species, consumers of horse meat and the environment at the recommended conditions of use.

The additive *Lactiplantibacillus plantarum* DSM 11520 is considered to be a respiratory sensitiser given its proteinaceous nature, but not irritant to skin or eyes in the product tested containing maltodextrin and oat bran as carriers. No conclusions can be drawn on the skin sensitisation potential of the additive.

*Lactiplantibacillus plantarum* DSM 11520 at the minimum recommended level of  $8.0 \times 10^{10}$  CFU/kg into oat-derived products (ca. 55% moisture content), carrot root-derived products ( $\geq 90\%$  moisture) and coconut flesh-derived products ( $\geq 90\%$  moisture) has the potential to reduce the pH of these feedingstuffs.

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<sup>27</sup> Annex\_IV\_1\_1 and Annex\_IV\_1\_1\_RFI-Oct22\_stats.

<sup>28</sup> Annex\_IV\_1\_2 and Annex\_IV\_1\_2\_RFI-Oct22\_stats.

<sup>29</sup> Annex\_IV\_1\_3 and Annex\_IV\_1\_3\_RFI-Oct22\_stats.



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## Abbreviations

CFU	colony forming unit
DM	dry matter
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
LOD	limit of detection
LOQ	limit of quantification

MIC                    minimum inhibitory concentration  
TTA                    total titratable acidity  
WGS                    whole genome sequence