SCIENTIFIC OPINION

Scientific opinion on the efficacy of *Lactobacillus plantarum* (ATCC 55058 and ATCC 55942) and *Pediococcus acidilactici* (CNCM I-3237) as silage additives for all animal species[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

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ABSTRACT

In 2010 the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of 18 strains of *Lactobacillus plantarum* and of six strains of *Pediococcus* spp. when used as feed additives for the production of silage. In those instances, however, due to limited and/or inconsistent evidence, the Panel was unable to draw conclusions on the efficacy of two strains of *L. plantarum* and one strain of *Pediococcus acidilactici*. The applicants have provided complementary information on the efficacy of these strains which is the subject of the current opinion. Three new studies were performed with each of the three strains under assessment. These involved laboratory-scale silos and samples of forage of differing water-soluble carbohydrate content. Replicate silos containing treated forage were compared to identical silos containing the same but untreated forage. Given the magnitude of the responses recorded and the absence of any substantive evidence of nutrient preservation, the data for the two *L. plantarum* strains, taken overall, provide little evidence of a benefit when used in the production of silage. The *P. acidilactici* strain has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile material by reducing the pH and increasing the preservation of dry matter at a minimum dose of 5×10^7 CFU/kg forage.

KEY WORDS

technological additive, silage additive, *Lactobacillus plantarum*, *Pediococcus acidilactici*, efficacy

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[1] On request from the European Commission, Questions No EFSA-Q-2013-00421 and EFSA-Q-2013-00436, adopted on 10 October 2013.

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SUMMARY

In 2010 the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued an opinion on the safety and efficacy of 18 strains of *Lactobacillus plantarum* and of six strains of *Pediococcus* spp. when used as feed additives for the production of silage. In those instances the Panel concluded that the strains were safe for target animals, consumers of animal products, users of the additive and the environment when used for the purpose described. However, due to limited and/or inconsistent evidence of a beneficial effect on the ensiling process, the FEEDAP Panel was unable to draw conclusions on the efficacy of two strains of *L. plantarum* and one strain of *Pediococcus acidilactici*.

The Commission has given the possibility to the applicants to submit complementary information to allow the Panel to reach conclusions on the efficacy of these strains. The further data provided are the subject of the current opinion.

Three additional studies were performed with each of the two *L. plantarum* strains and with the *P. acidilactici* strain. These involved laboratory-scale silos and samples of forage of differing water-soluble carbohydrate content. Replicate silos containing treated forage were compared to identical silos containing the same but untreated forage. Given the magnitude of the responses recorded and the absence of any substantive evidence of nutrient preservation, the data for the two *L. plantarum* strains, taken overall, provide little evidence of a benefit when used in the production of silage. The *P. acidilactici* strain has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile material by reducing the pH and increasing the preservation of dry matter at a minimum dose of 5×10^7 CFU/kg forage.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1831/2003[4] establishes rules governing the Community authorisation of additives for animal nutrition and in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant SILAC EEIG is seeking a Community authorisation of its *Lactobacillus plantarum* LP287/DSM5287/ATCC55058, *Lactobacillus plantarum* LP329/DSM5258/ATCC55942 and *Pediococcus acidilactici* CNCM I-3237, as silage additives (Table 1).

Table 1: Description of the substances

Category of additive	Technological additives
Functional group of additive	Silage additives
Trade name	-
Description	*Lactobacillus plantarum* LP287/DSM5287/ATCC55058, *Lactobacillus plantarum* LP329/DSM5258 /ATCC55942 and *Pediococcus acidilactici* CNCM I-3237
Target animal category	All animal species
Applicant	SILAC EEIG[5]
Type of request	Update opinion

On 23th May 2012, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority") in its opinions, was not able to give conclusive opinions on the efficacy of the products because of lack of data provided by the applicant.

Therefore, the Commission gave the possibility to the applicant to submit complementary information to complete the assessment on efficacy to allow a revision of that opinion.

The Commission has now received additional dossiers from the applicant, SILAC EEIG, on *Lactobacillus plantarum* LP287/DSM5287/ATCC55058, *Lactobacillus plantarum* LP329/DSM5258/ATCC55942 and *Pediococcus acidilactici* CNCM 1-3237 with supplementary information, concerning the efficacy as silage additives.

The Commission, in order to give the appropriate follow-up to the applications, asks the European Food Safety Authority to issue an updated opinion on the efficacy of these products under the terms of reference specified in the Annex. The data generated by the applicant and compiled in the above-mentioned supplementary reports have been sent directly to Authority by the applicant.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above, the Commission asks to the European Food Safety Authority to deliver an opinion on the efficacy of these *Lactobacillus plantarum* LP287/DSM5287/ATCC55058, *Lactobacillus plantarum* LP329/DSM5258/ATCC55942 and *Pediococcus acidilactici* CNCM 1-3237 for use in all animal species, as silage additives, taking into account the new information submitted.

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

5 On 13/03/2013, EFSA was informed by the applicant that SILAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures, representing notably the following companies: Pioneer Hi-Breed Inc. and Alltech.

ASSESSMENT

In 2010 a consortium of Companies made separate applications for the authorisation of 18 strains of *Lactobacillus plantarum* and six strains of *Pediococcus* spp. as additives for use in the production of silage (category: Technological additive, functional group: silage additives). EFSA assessed individually the strains under application for safety and for efficacy and concluded that all of the strains were safe for target animals, consumers of animal products, users of the additive and the environment when used for the purpose described (EFSA FEEDAP Panel, 2012a,b). However, *L. plantarum* strains ATCC 55058 and ATCC 55942 showed only limited and inconsistent evidence of a beneficial effect with difficult to ensile material with a low water soluble carbohydrate content (WSC). As there was no evidence that this led to the preservation of nutrients and as no data were provided for the use of these two strains with forage materials with a higher water-soluble carbohydrate content, the FEEDAP Panel was unable to draw conclusions on the efficacy of these two *L. plantarum* strains. Similarly, it was found that the data for *Pediococcus acidilactici* CNCM I-3237 were partly contradictory and inconsistent. Although some beneficial effects were shown in two studies, the opposite effect was shown in the third. Consequently, no conclusions on efficacy could be drawn.

Further data on the efficacy of these three strains have now been provided which are the subject of the current opinion.[6]

1. Conditions of use

The additional data provided did not specify conditions of use and so the information previously submitted is considered still to apply. The additives are intended for use with all forages at a proposed minimum dose shown in Table 1 and applied directly to silage (granular application) or as an aqueous suspension. However, it is noted that the application rates used in the additional studies are higher than the minimum dose shown in Table 1 and used in the earlier studies.

Table 1: Recommended dose for the three stains

Strain	Recommended dose (CFU/kg fresh silage)
L. plantarum ATCC 55942	5×10^6
L. plantarum ATCC 55058	5×10^6
P. acidilactici CNCM I-3237	1.4×10^7

2. Efficacy

In some studies, statistical significance was seen only when the second decimal point was considered. Generally, changes of this magnitude are considered of little or no biological relevance and so were not considered.

2.1. *Lactobacillus plantarum* ATCC 55942

New data from three laboratory experiments are described all made with forage maize samples with a target dry matter (DM) content of 35%. The experiments used 2.75 L mini-silos with the capacity to vent gas. In each case, the contents of four replicate silos were sprayed with the additive at 1.7×10^7 CFU/kg forage dissolved in water (not confirmed by analysis of the applied suspension). Forage for the control silos were sprayed with an equal volume of water but without the additive. Studies lasted for 90 days but no information was provided on storage conditions. The three additional studies involved forages of the same botanical origin and very similar WSC content, representing material easy to ensile (Table 2, studies 1 – 3). The earlier studies with this strain also involved forage maize, but with a low WSC content (Table 2, studies 4 – 6) and defined as difficult to ensile. These studies used a lower application rate (5×10^6 CFU/kg forage).

[6] Dossier references: FAD-2013-0001 and FAD-2013-0007.

Table 2: Forage maize used in the efficacy studies with *L. plantarum* ATCC 55942

Study	Forage	Dry matter content (%)	Water soluble carbohydrate content (% fresh matter)
1[7]	Maize forage	33.0	3.47
2	Maize forage	36.1	3.28
3	Maize forage	36.5	3.62
4	Maize forage	72.3	0.62
5	Maize forage	75.7	0.44
6	Maize forage	77.2	0.53

Replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acids concentration, alcohols and ammonia and total nitrogen in the earlier studies. Data from the additional studies were analysed within and across trials based on a model that included fixed effect of treatment and an error term. This was followed by pair-wise comparison of least-squares means of treatments with the respective controls based on a t-test of significance. The same methods were applied with the earlier data after tests for normality of distribution. The results of the new (studies 1 – 3) and previously submitted (studies 4 – 6) data are summarised in Table 3.

Table 3: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* ATCC 55942

Study	Dose (CFU/kg forage)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	0	1.9	3.5	0.8	0.3	nd
	1.7×10^7	1.7	3.4*	1.2*	0.3	
2	0	4.2	3.9	1.4	0.4	nd
	1.7×10^7	2.5	3.8*	1.6*	0.4	
3	0	3.8	3.8	1.2	0.4	nd
	1.7×10^7	2.8	3.8	1.4*	0.4	
4	0	2.1	4.0	0.7	0.1	3.7
	5×10^6	1.8*	4.0	0.8*	0.1	3.6
5	0	2.2	4.2	0.4	0	1.7
	5×10^6	2.5	4.2	0.5*	0	1.7
6	0	1.8	4.2	0.3	0	0.9
	5×10^6	2.3*	4.2	0.4*	0	1.1

*Significantly different from control value at $P < 0.05$.
nd: not determined.

The effects seen with both easy and difficult to ensile material are marginal. There was a significant increase in lactic acid content seen in all studies, but this translated into a reduction in final pH in only two studies. However, it should be noted that the pH in control silos was generally low leaving little margin for a further decrease. All three studies with easy to ensile material showed a numerical decrease in dry matter loss but this failed to reach significance. With difficult to ensile material dry matter loss was decreased in one study but increased in the other two. Given the magnitude of the responses recorded and the absence of any evidence of nutrient preservation, the data taken overall provides little evidence of a benefit.

[7] Technical dossier FAD-2013-0001.

2.2. *Lactobacillus plantarum* ATCC 55058

The additional data provided for *L. plantarum* ATCC 55058 was generated in the same series of experiments described previously for *L. plantarum* ATCC 55942 and made use of the same protocol and data from the same control silos. The results for these easy to ensile materials are shown in Table 4 as studies 1 – 3. Results from further four studies all made with difficult to ensile material were considered previously. Three of these studies made use of the same high dry matter forage maize samples used with *L. plantarum* ATCC 55942 and described in Table 2 (studies 4 – 6). Study 7 was made with an additional high dry matter maize sample (DM% 69.7, WSC 0.13%) and used only for *L. plantarum* ATCC 55058. As previously, replicate silos were opened at the end of the experiment and the contents were analysed for dry matter content, pH, lactic and volatile fatty acids concentration, alcohols and ammonia and total nitrogen in the earlier studies. The same statistical methods were used for analysis.

Table 4: Summary of the analysis of ensiled material recovered at the end of the experiments with *L. plantarum* ATCC 55058

Study	Dose (CFU/kg forage)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	Ammonia-N (% total N)
1	0	1.9	3.5	0.8	0.3	nd
	1.7×10^7	0.3	3.4*	1.1*	0.3	
2	0	4.2	3.9	1.4	0.4	nd
	1.7×10^7	2.5	3.8*	1.5*	0.4	
3	0	3.8	3.8	1.2	0.4	nd
	1.7×10^7	2.1	3.8	1.4*	0.4	
4	0	1.9	4.0	0.7	0.1	2.6
	5×10^6	2.0	4.0	0.6	0.1	2.6
5	0	2.2	4.2	0.4	0	1.7
	5×10^6	2.1	4.2	0.4	0	1.7
6	0	2.6	4.1	0.8	0.1	3.1
	5×10^6	2.4	4.1	0.8	0.1	2.8
7	0	2.2	4.1	0.6	0.1	3.1
	5×10^6	2.1	4.0*	0.6	0.1	2.6*

*Significantly different from control value at $P < 0.05$.
nd: not determined.

As seen with *L. plantarum* ATCC 55942, the effects of the additive when used with both easy and difficult to ensile material are marginal. Although there was a numerical decrease in dry matter loss in 6/7 studies, this failed to reach significance. Otherwise the few records of a significant decrease in pH or ammonia N as a fraction of total N were sporadic. The only consistent result was a small but significant increase in lactic acid content in all three studies with easy to ensile material. This was not replicated in the difficult to ensile material. Given the magnitude of the responses recorded and the absence of any significant evidence of nutrient preservation, the data taken overall provide little evidence of a benefit.

2.3. *Pediococcus acidilactici* CNCM I-3237

New data from three laboratory experiments are described all made with mixed swards of similar botanical composition (fully described in the dossier). The experiments used 4.5 L mini-silos with the capacity to vent gas. In each case, the contents of six replicate silos were sprayed with the additive at 5×10^7 CFU/kg forage or 1×10^8 CFU/kg forage dissolved in water (not confirmed by analysis of the applied suspension). Forage for the control silos were sprayed with an equal volume of water but without the additive. Studies lasted for 90 days and silos were held at $20 \pm 2^{o}C$. The three additional studies involved forages representing material easy, moderately difficult and difficult to ensile (Table

5, studies 1 – 3). The earlier studies with this strain involved ryegrass with a high WSC content and two legume samples with a low WSC content and defined as easy, moderately difficult and difficult to ensile (Table 5, studies 4 – 6). These studies used a lower application rate (1 – 3 x 10^7 CFU/kg forage). Thus the combined data set covers the full range of forage materials as defined in Regulation (EC) No 429/2008.

Table 5: Forage materials used in the efficacy studies with *P. acidilactici* CNCM I-3237

Study	Forage	Dry matter content (%)	Water soluble carbohydrate content (% fresh matter)
1[8]	Mixed sward	45.1	3.24
2	Mixed sward	34.2	1.60
3	Mixed sward	30.4	1.45
4	Perennial ryegrass	27.2	4.80
5	Red clover	15.0	1.52
6	Lucerne	18.7	1.46

Replicate silos were opened at the end of the 90 day experiments and the contents were analysed for dry matter content, pH, lactic and volatile fatty acids concentration, alcohols, ammonia and total nitrogen. Data were tested for normal distribution and when established analysed by a one-factorial completely randomised ANOVA design. In the few cases where a normal distribution could not be established a non-parametric analysis was used (Wilcoxon – Kruskal-Wallis). The results of the three newly submitted studies (studies 1 – 3) and the three original studies (studies 4 – 6) are summarised in Table 6.

Table 6: Summary of the analysis of ensiled material recovered at the end of the experiments with *P. acidilactici* CNCM I-3237

Study	Dose (CFU/kg forage)	Dry matter loss (%)	pH	Lactic acid (% ensiled matter)	Acetic acid (% ensiled matter)	$N\text{-}NH_3$ (% total N)
1	0	3.1	4.5	2.9	0.6	6.8
	5 × 10^7	2.6*	4.4*	3.2*	0.5*	6.5*
	1 x 10^8	2.3*	4.4*	3.2*	0.5*	6.4*
2	0	3.7	4.6	2.7	0.7	12.1
	5 × 10^7	2.2*	4.3*	2.9*	0.5*	8.8*
	1 x 10^8	2.1*	4.3*	2.9*	0.5*	9.2*
3	0	2.9	4.5	2.7	0.7	8.6
	5 × 10^7	2.6*	4.4*	2.8	0.7	8.8
	1 x 10^8	2.7*	4.4*	2.8	0.7	8.1
4	0	4.1	3.6	2.3	0.5	9.9
	1 x 10^7	10.4*	3.6	2.2	0.2*	9.9
5	0	17.5	3.8	1.5	0.3	13.5
	3 x 10^7	9.3*	3.8	1.6	0.2	13.4
6	0	17.1	6.2	0	0.3	23.1
	1 x 10^7	10.8*	5.1*	1.0*	0.6	22.6

*Significantly different from control value at $P < 0.05$.

[8] Technical dossier FAD-2013-0001.

There was a significant reduction in final pH in 4/6 studies. In the remaining two studies the pH of the control silos was low leaving little margin for a further reduction before low pH inhibited the growth and activity of lactic acid bacteria. The lowering of pH was accompanied by a reduced dry matter loss in 4/6 studies. There was also an indication of reduced protein breakdown although this reached significance in only two studies. Taking all studies into consideration *P. acidilactici* CNCM I-3237 has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile material at a minimum dose of 5 x 10^7 CFU/kg forage.

CONCLUSIONS

Given the magnitude of the responses recorded and the absence of any substantive evidence of nutrient preservation, the data for the two *L. plantarum* strains (ATCC 55942 and ATCC 55058) taken overall provides little evidence of a benefit when used in the production of silage.

P. acidilactici CNCM I-3237 has the potential to improve the production of silage from easy, moderately difficult and difficult to ensile material by reducing the pH and increasing the preservation of dry matter at a minimum dose of 5×10^7 CFU/kg forage.

DOCUMENTATION PROVIDED TO EFSA

1. Efficacy data for *Lactobacillus plantarum* LP287/DSM5287/ATCC55058 and LP329/DSM5258/ATCC55942 as silage additives. January 2013. Submitted by FEFANA Asbl.

2. Efficacy data for *Pediococcus acidilactici* CNCM I-3237 as silage additive. March 2013. Submitted by FEFANA Asbl.

REFERENCES

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012a. Scientific Opinion on the safety and efficacy of 18 strains of *Lactobacillus plantarum* (DSM 23375, CNCM I-3235, DSM 19457, DSM 16568, LMG 21295, DSM 16565, VTT E-78076, CNCM MA 18/5U, NCIMB 30238, ATTC PTA-6139, DSM 18112, ATCC 55058, DSM 18113, DSM 18114, ATCC 55942, ATCC 55943, ATCC 55944 and NCIMB 30094) as silage additives for all species. EFSA Journal;10(6):2732, 36 pp. doi:10.2903/j.efsa.2012.2732

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012b. Scientific Opinion on the safety and efficacy of *Pediococcus acidilactici* (CNCM I-3237, CNCM MA 18/5M—DSM 11673) and *Pediococcus pentosaceus* (DSM 23376, NCIMB 12455, NCIMB 30237 and NCIMB 30168) as silage additives for all species. EFSA Journal;10(6):2733, 15 pp. doi:10.2903/j.efsa.2012.2733