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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425) as a silage additive for all species[1]

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

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ABSTRACT

The mixture consisting of single strains of *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* is intended for use as a technological additive to improve the production of silage. Since growth medium is simultaneously inoculated with all three organisms, there is only limited control of the production system. Consequently, the qualitative composition of the additive mixture is only partially known at the point of production and may change further on storage. The species *S. cerevisiae*, *L. plantarum and L. casei* are considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. As the identity of all strains was clearly established and as no antibiotic resistance of concern was detected, the use of the strains in the production of silage is presumed safe for livestock, consumers of products from animals fed the treated silage and the environment. There is a potential for users to be exposed dermally and via inhalation with subsequent risks of skin and eye/respiratory irritation, and skin and respiratory sensitisation. The efficacy studies described provided no evidence that the additive containing *S. cerevisiae*, *L. plantarum* and *L. casei* has the potential to improve the production of silage. Although there was some evidence for an effect on aerobic stability, because of the inherent variability of the additive mix an effective dose could not be established.

KEY WORDS

silage additive, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Lactobacillus casei*, QPS, safety, efficacy

[1] On request from the European Commission, Question No EFSA-Q-2011-00390, adopted on 10 September 2013.

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SUMMARY

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 for the target animals, consumer, user and for the environment and on the efficacy of the product composed of a mixture of a yeast strain *Saccharomyces cerevisiae* and two bacterial strains, *Lactobacillus plantarum* and *Lactobacillus casei*, when used in combination as a technological additive intended to improve the ensiling process.

Since the production process involves growth medium simultaneously inoculated with all three organisms, there is only limited control of the production system with the result that the qualitative composition of the additive mixture is only partially known at the point of production and may change further on storage. The applicant declares a minimum content of "$>10^3$ CFU/mL" of additive for both the yeast and the lactic acid bacteria.

The species *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* are considered by EFSA to be suitable for the qualified presumption of safety approach to safety assessment. Therefore, strains belonging to these species do not require any specific demonstration of safety, other than confirming the susceptibility to antibiotics of human or veterinary clinical significance and the safety for the user. As the identity of all three strains was clearly established and as no antibiotic resistance of concern was detected in the bacterial species, the use of the strains in the production of silage is presumed safe for livestock, consumers of products from animals fed the treated silage and the environment.

Although users at the farm level are exposed to the additive for only a short period of time when preparing the aqueous suspension, because of the low pH of the product, there is a potential for skin and eye irritation. A potential for skin sensitisation is assumed. Since droplet/aerosol formation is unavoidable during the limited period the product is diluted and sprayed onto feed material, there is also a potential for exposure via an inhalation route and a consequent risk of irritation and sensitisation of the respiratory tract..

Six studies with laboratory-scale silos are described, each lasting at least 60 days, made using samples of grass forage of differing water-soluble carbohydrate content and representing material easy to ensile, moderately difficult to ensile and difficult to ensile. In each case, replicate silos containing treated forage were compared to identical silos containing the same but untreated forage. There was no evidence that the additive containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* has the potential to improve the production of silage. Although there was some evidence for an effect on aerobic stability, because of the inherent variability of the additive mix an effective dose could not be established.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular Article 10(2)/(7) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, within a maximum of seven years after the entry into force of this Regulation.

The European Commission received a request from the company EM Agriton B.V.[5] for re-evaluation of the product *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) *and Lactobacillus casei* (NBRC 3425) to be used as a feed additive for all animal species (category: technological additive; functional group: silage additive) under the conditions mentioned in Table 1.

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 10(2)/(7) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 1 June 2011.

This product was included in the European Union Register of Feed Additives following the provisions of Article 10(1) of Regulation (EC) No 1831/2003.

TERMS OF REFERENCE

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, consumer, user and the environment and the efficacy of the product *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) *and Lactobacillus casei* (NBRC 3425) when used under the conditions described in Table 1.

[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] EM Agriton B.V.Molenstraat 10-1, 8391 AJ Noordwolde. The Netherlands.
[6] EFSA Dossier reference: FAD-2010-0240.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	*Saccharomyces cerevisiae* NBRC 0203, *Lactobacillus plantarum* NBRC 3070 and *Lactobacillus casei* NBRC 3425
Registration number/EC No/No	-
Category(-ies) of additive	Technological
Functional group(s) of additive	Silage additive

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Saccharomyces cerevisiae NBRC 0203, *Lactobacillus plantarum* NBRC 3070 *Lactobacillus casei* NBRC 3425	-	-	ISO 21527:2008 ISO 15214:1998 ISO 15214:1998

Trade name	-
Name of the holder of authorisation	-

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period
		CFU/kg of complete feedingstuffs		
All species	-	-	-	

Other provisions and additional requirements for the labeling	
Specific conditions or restrictions for use	-
Specific conditions or restrictions for handling	-
Post-market monitoring	-
Specific conditions for use in complementary feedingstuffs	-

Maximum Residue Limit (MRL)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

Six genera of lactic acid producing bacteria are commonly associated with forage species and collectively contribute to the natural ensiling process. The present additive is a premix of three strains of microorganisms two of which are lactic acid bacteria (LAB) belonging to one of those six genera - *Lactobacillus plantarum* and *Lactobacillus casei*. The third organism is a strain of bakers/brewer's yeast *Saccharomyces cerevisiae*. They are intended to be added in combination to forages to promote ensiling (technological additive, functional group: silage additive) for the eventual use of the treated silage in all animals.

All three species are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007, EFSA BIOHAZ Panel, 2012). This approach requires the identity of the strains to be conclusively established and evidence that the two bacterial strains are susceptible to antibiotics of human and veterinary importance.

2. Characterisation

2.1. Identity and properties of the active agents

The strains are described as "collected from the natural environment" and all three are deposited in a Japanese culture collection (the National Institute of Technology and Evaluation (NITE) Biological Resource Centre).[7] *L. plantarum* has the accession number NBRC 3070 and is apparently synonymous with ATCC 8014. There appears some confusion over the identity of the strain deposited with the accession number NBRC 3425 which is also apparently synonymous with deposition ATCC 7469 described as *L. casei* subsp. *rhamnosus* and DSM 20021 described as *L. rhamnosus*. In the documentation the applicant sometimes describes the strain as *L. rhamnosus*. However, in the view of the FEEDAP Panel, strain identity of the two lactobacilli is established by the partial sequencing of the 16S rRNA and 23S rRNA genes and the complete 16S/23S intergenic spacer region which by comparison with sequences recorded in databases allowed the two organisms to be unambiguously identified as *L. plantarum* and *L. casei*. The strain of *S. cerevisiae* used has the accession number NBRC 0203 and was also indentified by molecular methods.[8] None of the strains has been genetically modified.

The applicant was asked to provide methods for the detection of the microbial strains specific to the additive, but chose to interpret this request in terms of identity which is not in question.[9] As a result, no methods for strain-specific detection or evidence of genetic stability have been made available.

The *Lactobacillus* strains were tested for antibiotic susceptibility using two-fold broth dilutions.[10] The battery of antibiotics tested included all of those recommended by EFSA (EFSA FEEDAP Panel, 2012). In the case of *L. plantarum* NBRC 3070, the cut-off value for kanamycin was exceeded by a single dilution. This is within the normal variation around a mean and is not considered to be a cause for concern by the FEEDAP Panel. In all other cases the minimum inhibitory concentration values for the two bacterial strains were equal or lower than the corresponding cut-off values defined by the FEEDAP Panel, and thus, no further investigation is required for these strains.

[7] Technical dossier/Section II/Annex 2.2.1.
[8] Technical dossier/Supplementary information January 2013/Annex ii.
[9] Technical dossier/Supplementary information January 2013/Annex ii.
[10] Technical dossier/Supplementary information October 2011 and January 2013/Annex Report MIC Free University Berlin and Annex i.

2.2. Production and characteristics of the additive

Very limited information on the manufacturing process is provided in the dossier. The applicant claims that the pH of the product is 3.5, however analyses of four batches revealed that the final pH of the product ranges between 2.8 and 3.5.[11] The additive appears to consist simply of the fermentation broth, possibly with the volume adjusted, and is stored and sold as a liquid product. The minimum specification given for the product is $>10^3$ CFU/mL *S. cerevisiae* and $>10^3$ CFU/mL total LAB. The ratio between the two strains of *Lactobacillus* is variable and therefore the applicant does not separately quantify the strains. Analysis of three batches of the additive showed counts of 1.5×10^4, 6×10^3 and 1.2×10^4 CFU/mL for *S. cerevisiae*. Numbers of lactobacilli were highly variable with values of 1.3×10^3, 3.5×10^3 and 3×10^6 CFU/mL.[12] An analysis of a fourth batch indicated that the count of lactobacilli in the product (1.3×10^2 CFU/mL) could be much lower.[13] However, in the absence of strain specific detection, it is not possible to establish if the counts of microorganisms in the final product are related to the product strains or to contaminants.

Data on microbial contamination was obtained from the time zero values of two batches of the additive used to assess the shelf-life of the product.[14] Other than yeast and lactic acid bacteria, numbers of other organisms were below the detection limits (*Escherichia coli* <10 CFU/mL; sulphite-reducing clostridia < 1 CFU/mL, *Bacillus cereus* < 10 CFU/mL; *Listeria monocytogenes* <10 CFU/mL and *Salmonella*, none in 25 g product).

Four batches of the additive were analysed for heavy metals and arsenic. Arsenic, mercury and cadmium were below detection limits (< 10, <0.5 and <1.0 µg/kg, respectively), copper was present with an average concentration of 28 µg/kg, iron at 11 µg/kg and lead at 15 µg/kg.[15] Given the nature of the fermentation medium the probability of contamination with mycotoxins is considered to be low and consequently not included in routine monitoring.

2.3. Stability

The additive is supplied in aqueous form and is intended for dilution at the point of use (see Section 2.4). Two batches of the additive were stored in their normal containers at 25 °C and 40 °C for a period of one year.[16] Samples were collected at regular intervals and numbers of yeasts and total LAB determined together with assays for a number of potential pathogenic bacteria (see Section 2.2). Numbers of yeasts remained essentially constant at 25 °C for the full duration of the experiment but significantly declined within 1-2 months at the higher temperature. At the lower temperature, numbers of LAB increased throughout the 12 month period and were some two orders of magnitude greater at the end of the study, possibly due to the molasses included in the formulation. The lactobaccilli were far less stable at 40 °C and were barely detectable at 12 months. The product would thus appear to have a shelf-life of about one year at temperatures not greater than 25 °C, but to be very sensitive to higher temperatures. In the absence of strain specific detection and because of the variable nature of the product it is not possible to comment on the individual stability of the two lactobacilli strains. As stated above, the lack of strain specific detection does not make it possible to establish whether the counts of microorganisms in the final product are related to the product strains or to contaminants. In addition, the extremely low pH of the product does not support the stability of a product containing viable microorganisms.

A short term (28 days) stability test made with a single batch of the product maintained at 25 °C confirmed that, despite the extremely low pH of 3.1, some growth of LAB was observed and that counts made at the end of the experiment were higher than those made initially.[17] When the

[11] Technical dossier/Section II and Supplementary information January 2013/Annexes 4.1.a, b, c and iii.
[12] Technical dossier/Section II/Annexes 4.1.a, b and c.
[13] Technical dossier/Supplementary information January 2013/Annex iii.
[14] Technical dossier/Section II/Annexes 2.4.1a, b and c.
[15] Technical dossier/Section II/Annex 2.1.4.
[16] Technical dossier/Section II/Annexes 2.4.1a and c.
[17] Technical dossier/Supplementary information January 2013/Annex iii.2.

concentrated additive was diluted to practical use concentrations (approximately 1 in 50), the pH was 3.9. Counts of LAB were <10 CFU/mL at the point of dilution but increased to 5 x 10^2 CFU/mL after 48 h under ambient conditions. No comparable counts were made of the yeast.

2.4. Conditions of use

The additive is intended for use with all forages and for all animal species. It is recommended that 2 L of the additive are diluted to 100 L with water and the diluted solution be applied by spraying to forage at an application rate of 4 L/tonne fresh material.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active agents in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

In the view of the FEEDAP Panel, the antibiotic susceptibility qualification has been met and the identity of the strains established. Consequently, the *L. plantarum*, *L. casei* and *S. cerevisisae* strains are considered suitable for the QPS approach to safety assessment. Therefore, no further assessment of safety, other than user safety, is required, and they are presumed safe for the target species, consumers of products from animals fed treated silage and the environment.

No specific studies in support of user safety are provided. However, the applicant recognises that, largely because of the low pH of the product, there is a potential for skin and eye irritation. A potential for skin sensitisation is assumed. Since droplet/aerosol formation is unavoidable during the limited period the product is diluted and sprayed onto feed material, there is also a potential for exposure via an inhalation route and a consequent risk of irritation and sensitisation of the respiratory tract. In the view of the FEEDAP Panel, the precautionary methods recommended in the material safety data sheet (MSDS) which accompanies the product should be sufficient to reduce the risk associated with these hazards. The FEEDAP Panel does consider the advice "Optional" applied to the use of protective gloves in the MSDS proposed by the applicant is weak and should be reconsidered.

Once an active agent has been authorised as a silage additive, different formulations can be placed on the market with reference to that authorisation. However, for assessing the safety for the user of the additive, the active agent is the principal focus provided that other components do not introduce concerns. The composition described in the dossier does not appear to contain other excipients.

4. Efficacy

4.1. Ensiling studies

A total of seven studies are described, some made with a single plant sample and others with a range of forage materials. However, in one case[18] the study was only of 52 days duration and the dose of additive applied was not stated. Although there were occasional significant differences between treated samples and their respective controls, these differences were not consistently seen either between samples or within a single forage sample. Consequently, this study is not further considered.

In five of the remaining six studies the additive was applied as a diluted solution which mimicked the recommended method of application (80 mL/tonne fresh material).

Studies 1 and 2 followed a common pattern and involved the forage materials shown in Table 2. Replicate 2.75 L mini-silos were used throughout with four replicates opened at various intervals during the studies and four to seven replicates opened at the end of the study (69 days for experiment

[18] Technical dossier/Section IV/Annex 4.1.1.

1a and 91 days for 1b and 79 days for study 2). Final silos were analysed for dry matter, pH, lactic acid, volatile fatty acids (VFA), ethanol, ammonia and total nitrogen. However acids were expressed as a percentage of total acidity and absolute amounts could not be calculated. A more limited analysis was made for the intermediate samples. At the end of the experiment an estimate of aerobic stability was then made using the Honig method (Honig, 1986).

In these and the other studies considered normality of data was tested by Kolmogorov-Smirnov and equality of variances by Levene's test. Normally distributed, homoscedastic data were subjected to two-sided one way ANOVA with Tukey as *post hoc* test. Otherwise, data were subjected to two-sided non-parametric one way ANOVA according to Wilcoxon (using Bonferroni correction). Significance was declared at $p < 0.05$. Results are summarised in Table 3.

Table 2: Characteristics of the forage samples used in the six studies performed

Study No	Test material	Dry matter content (%)	Water soluble carbohydrate content (% fresh matter)
1a[19]	Ryegrass + white clover (second cut)	32.6	3.2
1b[20]	Ryegrass + white clover (third cut)	31.3	2.2
2[21]	Whole crop maize (*cv* Lafortuna)	31.8	1.3
3a[22]	*Lolium perenne* (first cut wilted)	38.5	6.6
3b[23]	*Lolium perenne* (first cut wilted)	40.6	7.5
3c[24]	*Lolium perenne* (first cut wilted)	43.1	12.6
4[25]	Whole crop maize	36.0	-
5[26]	Whole crop maize	33.9	-
6[27]	*Lolium multiflorum* (second cut)	35.9	3.2

Study 3 consisted of three separate experiments each of 90 days duration and each using cuts of "pasture grass" consisting largely of *Lolium perenne*. The three experiments followed a common design in which four replicate 1 L jars per treatment were filled and kept at room temperature before opening at the end of the experiment and the contents analysed. In addition, four replicate plastic bags/treatment containing 2 kg of the grass samples were similarly treated and three used after 90 days for the estimate of aerobic stability (loss of stability defined as a 3°C rise in temperature). The results are summarised in Table 3.

Study 4 was predominately concerned with effects on temperature rise after clamp opening (aerobic stability) and involved the comparison of a control with a number of commercial products including an organic acid. Five replicate mini-silos (size not stated) were used for each treatment. However the

[19] Technical dossier/Section IV /Annex4.1.2 .
[20] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4.1.2 and v.1.
[21] Technical dossier/Section IV/Annex 4.1.3.
[22] Technical dossier/Section IV/Annex 4.1.4a.
[23] Technical dossier/Section IV/Annex 4.1.4b.
[24] Technical dossier/Section IV/Annex 4.1.4c.
[25] Technical dossier/Section IV/Annex 4.1.5.
[26] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4.1.6 and v.2.
[27] Technical dossier/Section IV and Supplementary information January 2013/Annexes 4.1.7 and v.3.

dose applied (described as 1 x 10^8 CFU/kg maize) cannot be related to the recommended dose. After 47 days some air was allowed into the silos which were then emptied after 62 days. Thereafter aerobic stability was measured for the next 7 days following the Honig method (Honig, 1986) (Table 3).

Studies 5 and 6 also examined aerobic stability after treatment with the additive following the Honig method (Honig, 1986). In study 5 five replicate silos (2.75 L) per treatment filled with whole crop maize (Table 2) were challenged by partial opening after 44 days and then emptied after 62 days. Thereafter aerobic stability was followed for seven days with a rise of 3°C taken to indicate loss of stability. Although organic acids were measured, results were expressed only as a percentage of total acidity. In the case of study 6 the material ensiled was Italian ryegrass and six replicates were used per treatment. In this case the silage was challenged after 85 and 106 days ensiling and the silos finally emptied after 124 days followed by the test for aerobic stability.

Table 3: Summary of the analysis of ensiled material recovered at the end of the experiments from the six studies made with the additive

Study No (Duration, days)	Group	Dry matter loss (%)	pH	Lactic acid (% ensiled material)	Acetic acid (% ensiled material)	Ammonia-N (% total N)	Aerobic stability (days)
1a (69)	Control	1.5	4.5	-	-	7.9	-
	Treated	2.0*	4.3*	-	-	6.3*	-
1b (91)	Control	0.9	4.4	-	-	8.0	2.7
	Treated	1.7*	4.7*	-	-	7.8*	>7.8*
2 (79)	Control	0.9	3.9	-	-	4.4	0.9
	Treated	0.9	3.8*	-	-	4.8	1.3
3a (90)	Control	22.7	4.1	3.8	0.4	4.0	7.7
	Treated	21.8	4.1	3.9	0.3	3.7	7.2
3b (90)	Control	18.5	4.1	4.2	0.3	3.7	5.7
	Treated	19.2	4.1	4.3	0.3	5.0*	7.1
3c (90)	Control	15.9	4.1	3.8	0.2	5.3	5.3
	Treated	15.8	4.1	3.8	0.2	2.3	6.7
4 (62)	Control	2.2	4.2	0.7	0.5	-	1.7
	Treated	2.2	4.1	0.6	0.4	-	2.9
5 (62)	Control	1.3	3.7	-	-	4.1	1.8
	Treated	1.3	3.8	-	-	4.0	2.6*
6 (124)	Control	2.1	4.4	1.0	0.5	-	1.8
	Treated	2.3	3.9*	1.7*	1.2*	-	>8.3*

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

Overall there is no evidence of a consistent beneficial effect on the quality of silage produced which might arise from the presence of the additive. Results for aerobic stability were more consistent with the additive appearing in most studies to increase the period before deterioration occurred. Differences reached significance in three trials, although in one the magnitude of the improvement was less than 24 h.

4.2. Effective composition and dose

It is evident from Section 2.2 that there is only limited control of the production system with the result that the qualitative composition of the additive mixture is only partially known at the point of production and may change further on storage. The applicant declares a minimum content of "$>10^3$ CFU/mL" of additive for both the yeast and the LAB. This represents a range between marginally

more than 1 x 10^3 CFU and infinity but, in practice, sets a minimum declared content of 10^3 CFU/mL for the yeast and for the LAB mixture.

Applying this minimum specified microbial content of the additive and the dilution scheme recommended by the applicant (see 2.4) the additive would deliver 80 CFU of the yeast and 80 CFU lactobacilli/kg fresh material. There is no suggestion that the additive is a "grow-up" product and so it has to be assumed that this is the minimum intended dose. The Panel considers that an inoculum of this magnitude would have no effect on ensiling as the number of cells added is several orders of magnitude lower than the level of natural microbiota of the material to be ensiled.

Analysis of three production batches (Section 2.2) and description given of the additive in the efficacy studies, when this was available, suggests that the counts made of yeast cells were fairly consistent and related to the declared minimum content allowing for reasonable overage. The mean value for the three production batches was 1.1 x 10^4 CFU/mL and 1 x 10^3 CFU/mL for the additive batches used in the efficacy studies. However there was one batch used in an efficacy study (6) in which no yeast could be detected.

Counts made of LAB in batches of additive used in the efficacy studies were highly variable ranging from 1 x 10^4 CFU/mL to 1.8 x 10^8 CFU/mL, greater than four orders of magnitude. Although there was some indication of increased aerobic stability in three of the eight studies provided where this was measured, the LAB counts in the additive applied were 1.8 x 10^8 CFU/mL in the case of study 1b, 3.8 x10^4 CFU/mL in study 6 and not given in study 5. Consequently, it is not possible to establish a minimum effective dose.

CONCLUSIONS

The very limited control applied to the production process results in an additive mixture whose qualitative composition can only be presumed and whose quantitative composition cannot be predicted and may change further on storage.

The identity of the strains of *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* has been confirmed and no antibiotic resistance was detected in the bacterial strains. Consequently, following the QPS approach to safety assessment, the use of these strains alone or in combination in the production of silage is considered safe for all animal species, consumers of products from animals fed treated silage and for the environment.

Users at the farm level are exposed to silage additive for only a short period of time when preparing the aqueous suspension. The FEEDAP Panel considers that there is a potential for skin and eye irritation because of the low pH of the product. A potential for skin sensitisation is assumed. Since droplet/aerosol formation is unavoidable during the limited period the product is diluted and sprayed onto feed material, there is also a potential for exposure via a inhalation route and a consequent risk of irritation and sensitisation of the respiratory tract

The minimum specification for the additive, when used as recommended, does not allow for a measurable effect on ensiling or the aerobic stability of ensiled material. From the studies performed with a diluted solution of the additive which mimicked the recommended method of application, there is no evidence that the additive containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Lactobacillus casei* has the potential to improve the production of silage. Although there was some evidence for an effect on aerobic stability, an effective dose cannot be established.

DOCUMENTATION PROVIDED TO EFSA

1. *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425). November 2010. Submitted by EM Agriton B.V.

2. *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425). Supplementary information October 2011. Submitted by EM Agriton B.V.

3. *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425). Supplementary information January 2013. Submitted by EM Agriton B.V.

4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425) for all animal species.

5. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of *Saccharomyces cerevisiae* (NBRC 0203), *Lactobacillus plantarum* (NBRC 3070) and *Lactobacillus casei* (NBRC 3425) for all animal species[28]

This report is on the evaluation of feed additives "*micro-organisms used as silage agents*", which is related to the application of (1) forty two *micro-organisms* for which authorisation is sought under Article 10(2) and (2) three additional *micro-organisms* for which authorisation is sought under Article 4(1). Authorisation is sought for all the above mentioned *micro-organisms* under category/functional group 1(k), technological additives/silage additives, according to Annex I of Regulation (EC) No 1831/2003. The list of *micro-organisms* of interest and the minimum activities in the *feed additives* and in *silage*, as sought in the authorisation, are presented in Table 1.[29] The intended use of the current applications is for all animal species, except for FAD-2011-0001, for which pigs, bovines, sheep, goats and horses are specified.

For identification and characterisation of *Saccharomyces cerevisiae* the EURL recommends for official control Polymerase Chain Reaction (PCR), a generally recognised standard methodology for identification of yeasts. For identification and characterisation of all the other micro-organisms of concern (i.e. *lactococci*, *lactobacilli*, *pediococci* and *bacilli*) the EURL recommends for official control Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for microbial identification.

The EURL recommends for enumeration in the *feed additives* the following ring trial validated methods:

- Pour plate method using MRS agar (ISO 15214) for *Lactococci;*
- Spread plate method using MRS agar (EN 15787) for *Lactobacilli*;
- Spread plate method using MRS agar (EN 15786) for *Pediococci;*
- Spread plate method using tryptone soya agar (EN 15784) for *Bacilli;* and
- Pour plate method using CGYE agar (EN 15789) for *Saccharomyces.*

None of the Applicants provide experimental data for the determination of *micro-organisms* in *silage*. Furthermore, the unambiguous determination of the content of micro-organisms added to *silage* is not achievable by analysis. Therefore the EURL cannot evaluate nor recommend any method for official control to determine any of the forty five *micro-organisms* of concern in *silage*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

[28] The EURL produced a combined report for *Lactococci, Lactobacilli, Pediococci, Bacilli* and *Saccharomyces.*
[29] Full list provided in EURL evaluation report, available from the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-uorg-silage-group1.pdf