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Safety and efficacy of Lavipan[®] (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) for weaned piglets, chickens for fattening and turkeys for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

Following a request from the European Commission, the EFSA 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 Lavipan[®] (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) when used as a zootechnical additive for weaned piglets, chickens and turkeys for fattening at the minimum dose of 5×10^8 colony forming unit (CFU) lactic acid bacteria (LAB)/kg feed and 5×10^6 CFU *S. cerevisiae*/kg feed. The species *Lc. lactis*, *C. divergens*, *Lb. casei*, *Lb. plantarum* and *S. cerevisiae* are considered by EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment and not to require specific demonstration of safety other than demonstrating the absence of resistance to antibiotics of human and veterinary significance. The identity of all strains has been established and no antibiotic resistance of concern detected. Following the QPS approach to safety assessment, these strains are presumed safe for the target species, consumers of products from animals fed the additive and the environment. Lavipan[®] is not toxic by inhalation or a dermal/ocular irritant, but should be considered as a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the skin sensitisation potential. Lavipan[®] has the potential to improve the performance of chickens for fattening when supplemented at the recommended dose of 5×10^8 CFU LAB/kg feed and 5×10^6 CFU *S. cerevisiae*/kg feed. No conclusions can be drawn on the efficacy of Lavipan[®] when added to feed for weaned piglets or turkeys for fattening. Lavipan[®] is compatible with diclazuril, salinomycin sodium, decoquinat, maduramicin and narasin+nicarbazin.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 JHJ Ltd² for the authorisation of the product Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p),³ when used as a feed additive for piglets (weaned), chickens for fattening and turkeys for fattening (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 dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 31 July 2014.

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 Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The additive Lavipan® is a preparation containing viable cells of *Lc. lactis* B/00039, *C. divergens* KKP 2012p, *Lb. casei* B/00080, *Lb. plantarum* B/00081 and *S. cerevisiae* KKP 2059p. It has not been previously authorised as a feed additive in the European Union (EU).

The species *Lc. lactis*, *Lb. casei*, *Lb. plantarum*, *C. divergens* and *S. cerevisiae* are considered by the EFSA to be suitable for the qualified presumption of safety (QPS) approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2013, 2014). This approach requires the identity of the strain to be conclusively established and evidence that the strain does not show resistance to antibiotics of human and veterinary importance.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier⁴ in support of the authorisation request for the use of Lavipan® as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008⁵ and the applicable EFSA guidance documents.

EFSA has verified the European Union Reference Laboratory (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 Annex A.

¹ 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.

² JHJ LTD. Nowa Wieś 11, 63–308, Gizałki, Poland.

³ In the mandate, the applicant identified the strains with in-house identifiers: *Lactococcus lactis* IBB500, *Carnobacterium divergens* S1, *Lactobacillus casei* ŁOCK 0915, *Lactobacillus plantarum* ŁOCK 0862 and *Saccharomyces cerevisiae* ŁOCK 0141.

⁴ FEED dossier reference: FAD-2013-0048.

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.

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Lavipan® 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, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Technical Guidance: extrapolation of data from major species to minor species regarding the assessment of additives for use in animal nutrition (EFSA, 2008a), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2012c), and Technical guidance – compatibility of zootechnical microbial additives with other additives showing antimicrobial activity (EFSA, 2008b).

3. Assessment

The additive Lavipan® is a preparation containing viable cells of *Lc. lactis* B/00039, *C. divergens* KKP 2012p, *Lb. casei* B/00080, *Lb. plantarum* B/00081 and *S. cerevisiae* KKP 2059p intended for use as a feed additive for weaned piglets, and chickens and turkeys for fattening (category: zootechnical additives; functional group: gut flora stabilisers).

3.1. Characterisation

3.1.1. Characterisation of the active agents

The *Lc. lactis* strain was isolated from cow's milk and is deposited in the Polish Collection of Microorganisms (PCM) with the accession number B/00039.⁶ The *C. divergens* strain was isolated from the digestive system of a common carp and is deposited in the Polish Collection of Industrial Microorganisms with the accession number KKP 2012p.⁷ The *Lc. casei* strain, isolated from milk fermented drinks, and the *Lb. plantarum* strain, isolated from silage, are deposited in the PCM with the accession numbers B/00080 (*Lc. casei*)⁸ and B/00081 (*Lb. plantarum*),⁹ respectively. The *S. cerevisiae* strain, isolated from silage, is deposited in the Polish culture Collection of Industrial Microorganisms with the accession number KKP 2059p.¹⁰

The bacterial strains were identified using the complete sequence of 16S rRNA gene and species specific primers pairs while the *S. cerevisiae* by means of the internal transcribed spacer region sequence.¹¹

The bacterial strains were tested for antibiotic susceptibility using commercial agar diffusion techniques. The battery of antibiotics used included those recommended by EFSA (EFSA FEEDAP Panel, 2012c).¹² The minimum inhibitory concentration (MIC) values for the *C. divergens* strain were compared to the cut-off values for facultative heterofermentative *Lactobacillus* which is considered by the Panel as the closest group documented in the Guidance. The MIC values for all strains were below or equal to the EFSA cut-off values with the exception of *Lc. lactis* for which the MIC for streptomycin was exceeded by a single dilution. This is within the normal variation around the mean, and thus, does not raise concerns for safety.

3.1.2. Manufacturing process and characterisation of the additive¹³

The manufacturing process is detailed in the dossier.

⁶ Technical dossier/Section II/Annex II_19.

⁷ Technical dossier/Section II/Annex II_20.

⁸ Technical dossier/Supplementary information May 2015/Annex 1/Annex 1.II.

⁹ Technical dossier/Supplementary information May 2015/Annex 1/Annex 1.I.

¹⁰ Technical dossier/Supplementary information May 2015/Annex 1/Annex 1.III.

¹¹ Technical dossier/Section II/Annexes II_23-27.

¹² Technical dossier/Supplementary information May 2015/Annex 2.

¹³ This section has been amended following the confidentiality claims made by the applicant.

The applicant declares a minimum specification of 1×10^9 colony forming unit (CFU) lactic acid bacteria (LAB)/g and $> 1 \times 10^7$ CFU *S. cerevisiae*/g and specifically:¹⁴

- *Lc. lactis* $\geq 5 \times 10^8$ CFU/g
- *Lb. plantarum* $\geq 3 \times 10^8$ CFU/g
- *Lb. casei* $\geq 1 \times 10^8$ CFU/g
- *C. divergens* $\geq 3 \times 10^8$ CFU/g
- *S. cerevisiae* $\geq 1 \times 10^7$ CFU/g

Batch-to-batch variation tested on six batches and based on individual counts showed that specifications were always exceeded:¹⁵

- *Lc. lactis*: mean = 4.4×10^9 CFU/g, range = $1.0\text{--}13 \times 10^9$ CFU/g
- *Lb. plantarum*: mean = 3.3×10^9 CFU/g, range = $1.1\text{--}13 \times 10^9$ CFU/g
- *Lb. casei*: mean = 3.7×10^9 CFU/g, range = $1.1\text{--}12 \times 10^9$ CFU/g
- *C. divergens*: mean = 3.3×10^9 CFU/g, range = $1.1\text{--}12 \times 10^9$ CFU/g
- *S. cerevisiae*: mean = 3×10^9 CFU/g, range = $1\text{--}4 \times 10^9$ CFU/g

Tests of additional three batches showed compliance with specifications based on total lactobacilli and yeast counts.¹⁶

Certificates of analysis of at least three batches of the additive for chemical contaminants (aflatoxins B1, B2, G1, G2, zearalenone, ochratoxin A, deoxynivalenol, heavy metals and arsenic) and for microbiological contaminants were submitted. Action limits have been set for lead (< 10 mg/kg), cadmium (< 0.5 mg/kg), mercury (< 0.1 mg/kg), arsenic (< 4 mg/kg), filamentous fungi (< 100 CFU/g), *Escherichia coli* (absence in 1 g) and *Salmonella* (absence in 25 g). Analyses demonstrated compliance with these limits.¹⁷ No specifications were set for mycotoxins. Levels found were all lower than the limit of detection (LOD) of the methods for aflatoxins B1, B2, G1 and G2 and zearalenone.¹⁸ For deoxynivalenol, two batches were below the LOD,¹⁹ and in one case, the value measured was $24.3 \mu\text{g/kg}$. For ochratoxin A, analysis showed values of $1.10 \mu\text{g/kg}$, $1.60 \mu\text{g/kg}$ and $< 0.12 \mu\text{g/kg}$ (LOD).¹⁷

One batch of Lavipan® was examined for particle size distribution by laser diffraction and dusting potential with a Heubach dustometer.²⁰ Results showed that 4.4% by volume of the additive consists of particles with diameters below $50 \mu\text{m}$ and no particles with diameter below $10 \mu\text{m}$ were present. The dusting potential was 0.01 g/m^3 .

3.1.3. Stability and homogeneity

3.1.3.1. Shelf life

The shelf life of each separate strain composing the additive (apparently one batch of each, 12 replicates) was tested at two conditions (4°C /relative humidity (RH) 35% and 20°C /RH 50%) for 12 months.²¹ Bacterial counts' losses were < 0.5 log over the full experimental period when samples were retained in its original packaging at 20°C . In case of the yeast counts, 1 log reduction was observed at 20°C /RH 50% after 1 month. Data on the individual strains is considered to represent their behaviour when included in the additive.

3.1.3.2. Stability in premixtures and feedingstuffs

The applicant declares that Lavipan® is not suitable for use in vitamins/minerals premixtures.²²

To test the stability in mash feed, a study with feed for turkeys was conducted including three batches of a mixture of the strains composing Lavipan® in a proportion mimicking its composition.²³ The samples were stored at two conditions (4°C /RH 50% and 20°C /RH 35%), and LAB and yeasts counts made at 2 week intervals for up to 3 months. The total counts of LAB remained constant

¹⁴ Technical dossier/Supplementary information May 2015/Annex 3.

¹⁵ Technical dossier/Section II/Annex II_2.

¹⁶ Technical dossier/Section II/Annex II_3.

¹⁷ Technical dossier/Section II/Annex II_4-18.

¹⁸ Limits of detection: for aflatoxins B1, B2, G1 and G2: $< 0.21 \mu\text{g/kg}$ and for zearalenone: $< 0.78 \mu\text{g/kg}$.

¹⁹ Limit of detection for deoxynivalenol $< 4.33 \mu\text{g/kg}$.

²⁰ Technical dossier/Supplementary information May 2015/Annexes 4.

²¹ Technical dossier/Supplementary information May 2015/Annex 6.

²² Technical dossier/Supplementary information May 2015/Annex 7.

²³ Technical dossier/Supplementary information November 2015/Annex 1.

(losses < 0.5 log) in all three batches when the product was stored at 20°C/RH 35% up to 4 weeks, while more than 1 log reduction was observed after 12 weeks. All the data presented refer to total counts of LAB and not to the single strains. Considering the short shelf life of the additive, the FEEDAP Panel does not expect relevant differences among the bacterial strains. Viable cell counts of the yeast remained unchanged (losses < 0.5 log) at 4°C/RH 50% over 8 weeks and at 20°C/RH 35% over 2 weeks, while a 2 log reduction was observed after 12 weeks.

The stability of the mix of the four LAB strains and the yeast strain (three batches) to pelleting of feed (composition not described) was measured. Counts of total LAB and yeasts in feed were determined before and after the pelleting process at 60, 70 and 80°C.²⁴ Although the total counts were not significantly affected by the heat treatment (losses < 0.5 log), survivability of the individual bacterial strains was not determined. Counts of viable cells of the yeast strain remained unchanged (losses ≤ 0.5 log) after treatment at all conditions.

A study was conducted with feed for piglets, mixed with the individual cultures in a proportion in accordance with the manufacturing process of Lavipan® and the resulting mixture was subject to pelleting (75°C).²⁵ The pelleted feed was stored at two conditions (4°C/RH 50% and 20°C/RH 35%) over 3 months. The counts in the pelleted feed remained unchanged (losses < 0.5 log) at 4°C over 12 weeks. In contrast, at 20°C during the first 4 weeks, the counts of viable cells decreased for at least 1 log for all strains except *Lc. lactis*. In an additional study, stability of the complete additive (three batches) mixed with pelleted feed for turkeys was monitored during storage at 4°C/RH 50% and 20°C/RH 35%.²⁶ In this case, counts of total LAB and of yeast were made over a 12 week period. Counts of LAB remained unchanged (losses < 0.5 log) at 4°C/RH 50% over 12 weeks and at 20°C/RH 35% over 4 weeks. Counts of the yeast remained unchanged (losses < 0.5 log) at 4°C/RH 50% over 8 weeks and at 20°C/RH 35% over 4 weeks.

Overall, although the additive appears to resist pelleting, stability in complete feed (mash or pelleted) can only be assured for 3 months when the feed is stored at 4°C.

3.1.3.3. Homogeneity

The capacity of Lavipan® to homogeneously mix with feed was measured in three batches of a feed (composition not provided). Each batch was divided into 10 subsamples.²⁷ Cell counts showed a coefficient of variation (CV) < 15% for both total LAB and yeasts.

3.1.4. Conditions of use

Lavipan® is intended to be used in diets for weaned piglets, chickens for fattening and turkeys for fattening at the minimum dose of 500 mg/kg feed equating to 5 x 10⁸ CFU LAB/kg feed and 5 x 10⁶ CFU *S. cerevisiae*/kg feed.

Lavipan® is not suitable for use in vitamins/minerals premixtures.

The applicant intends to use Lavipan® in conjunction with some coccidiostats (diclazuril, salinomycin sodium, decoquinate, maduramicin and narasin+nicarbazin).

3.2. Safety

3.2.1. Safety for the target species, the consumer and the environment

In the view of the FEEDAP Panel, the identity of the strains of *Lc. lactis*, *Lb. plantarum*, *Lb. casei*, *C. divergens* and *S. cerevisiae* has been established and the antibiotic resistance qualification of the bacterial strains has been met. Accordingly, these strains are considered by EFSA to be suitable for the QPS approach to safety assessment (EFSA, 2007; EFSA BIOHAZ Panel, 2013, 2014) and are presumed safe for the target species, consumers of products from animals fed the additive and the environment.

3.2.2. Safety for the user

An acute inhalation toxicity study on rats was performed according to Organisation for Economic Co-operation and Development (OECD) Technical Guideline 403.²⁸ Wistar rats were exposed to the

²⁴ Technical dossier/Supplementary information November 2015/Annex 3.

²⁵ Technical dossier/Supplementary information May 2015/Annex 8.

²⁶ Technical dossier/Supplementary information November 2015/Annex 2.

²⁷ Technical dossier/Supplementary information November 2015/Annex 4.

²⁸ Technical dossier/Supplementary information May 2015/Annex 10/Annex 10_IV.

additive at the highest attainable concentration, i.e. 0.34 mg/L for 4 h in a nose-only inhalation chamber. No clinical signs were observed in any of the animals after a 14 day-observation period. This result indicated that the test material was not toxic by inhalation.

A small fraction of the product ($\geq 4\%$) has a diameter below 50 μm , and although the dusting potential is low, there is a potential exposure of the upper respiratory tract. Given the proteinaceous nature of the active agents, the additive should be considered to be a potential respiratory sensitiser.

Lavipan® was tested in studies of irritancy to skin of Wistar rats and to eyes of New Zealand White rabbits according to OECD Technical Guidelines 402 and 405, respectively. The results of the studies of skin irritancy gave no indication that the additive causes irritation to skin.²⁹ In the eye irritancy study, Lavipan® caused an initial reddening of the conjunctiva followed by some swelling of the conjunctiva and moderate discharge from the eye that disappeared after 48 h.³⁰ This result indicated that the test material was not irritant to eye.

No data are available on skin sensitisation for Lavipan®.

3.2.2.1. Conclusions on safety for the user

Lavipan® is not toxic by inhalation or a dermal/ocular irritant, but should be considered as a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the skin sensitisation potential.

3.3. Efficacy

3.3.1. Efficacy for weaned piglets

Overall six studies with weaned piglets, conducted in the same Member State but in different locations were submitted. One study³¹ was disregarded because the initial weight of the control group was significantly smaller than that of the treated animals, introducing a bias in the study. Other three studies,³² with a very similar experimental design, were submitted upon detailed request. However, the detailed information needed to fully assess the studies was not provided (e.g. absence of overall feed intake data, absence of the average initial and final weights, uncertainty about experimental unit (pig or pen), inadequate statistical reporting). Therefore, the FEEDAP Panel was unable to conclude on these studies. The remaining two studies are described below.

The first of the two remaining studies involved 96 weaned piglets (average age 31 days, average initial body weight (bw) 8.2 kg, synthetic line) distributed in two treatment groups (control and Lavipan®).³³ Each group was replicated six times, each pen involving eight piglets (four males and four females). The trial lasted 42 days during which the piglets received a basal prestarter diet for 14 days and a growing diet for the following 28 days, *ad libitum*. The diet of the Lavipan® group was supplemented with the additive at the minimum recommended dose (5×10^8 CFU LAB/kg feed and 5×10^6 CFU *S. cerevisiae*/kg feed, confirmed by analysis). Individual body weight at the start and at 14 and 42 days after weaning, feed intake per pen and per feeding phase and overall were measured, and average daily gain and feed to gain ratio were also calculated. Morbidity and mortality were also monitored. The data were analysed with analysis of variance (ANOVA) using age and initial weight as covariates.

One animal in the Lavipan® group died. No significant differences on the performance of piglets were observed. The final body weight was ~ 24.5 kg and the average weight gain was ~ 385 g/pig/day. Despite the request, no data on feed intake or feed to gain ratio were provided for the overall period.

The second study involved 256 piglets (27 days of age, average initial body weight 7.8 kg, synthetic line) randomly allocated according to sex and weight to four treatment groups, each replicated eight times (each replicate consisting of four males and four females).³⁴ The experimental groups resulted from the supplementation of the basal diet with Lavipan® at the intended doses of 5×10^7 , 5×10^8 and 5×10^9 CFU/kg. Although it was not specified what these counts refer to, it is assumed they refer to total LAB. Piglets were fed *ad libitum* a prestarter and starter diet based on barley/wheat/maize, in pelleted form. The trial lasted 49 days. The parameters measured were body

²⁹ Technical dossier/Supplementary information May 2015/Annex 10/Annex 10_III.

³⁰ Technical dossier/Supplementary information May 2015/Annex 10/Annex 10_I.

³¹ Technical dossier/Supplementary information May 2015/Annex 11 i 12/Annex 1112I.

³² Technical dossier/Supplementary information November 2015/Annexes 5.1, 5.2 and 5.3.

³³ Technical dossier/Section IV/Annex IV.1.

³⁴ Technical dossier/Supplementary information May 2015/Annex 11 i 12/Annex 1112II.

weight (on days 0, 14, and 49 days) and feed intake per pen. Morbidity and mortality were monitored and average daily gain and feed to gain ratio (per pen) calculated. The data were subject to ANOVA with initial weights as covariant.

Mortality and culling were low (3%) and apparently were not influenced by treatment (one animal from the control was removed, one from the 5.4×10^7 CFU/kg feed group died, two from the 5.2×10^8 CFU/kg feed group died and from the group at 4.8×10^9 CFU/kg feed, three were excluded and one died). No significant differences in performance were found in the overall period. The final body weight was ~ 30 kg, average daily weight gain ~ 460 g/pig per day, daily feed intake ~ 780 g/pig per day and feed to gain ratio ~ 1.7. Use of a single value for CFUs does not relate to the specifications provided for the additive.

3.3.1.1. Conclusions on efficacy for weaned piglets

None of the studies provided showed an effect of the additive on the performance of the animals.

3.3.2. Efficacy for chickens for fattening

Four studies with chickens for fattening conducted in the same Member State but in two different locations were presented.

In all cases, 1-day-old Ross 308 birds (females and males 1:1 in studies 1³⁵ and 2,³⁶ males in study 3³⁷ and females in study 4³⁸) were fed *ad libitum* mash diets based on wheat/maize/soybean meal, containing salinomycin during an experimental period of 35–42 days. Studies considered two or more experimental groups, one receiving the basal diet not supplemented with the additive and the others receiving the same basal diet supplemented with the additive at different inclusion rates. Study 1 included one Lavipan® group receiving the diet supplemented with the additive at the recommended dose (5×10^8 CFU LAB/kg feed and 5×10^6 CFU *S. cerevisiae*/kg feed). Study 2 included two diets supplemented with Lavipan® to levels of LAB/*S. cerevisiae* of $5 \times 10^7/5 \times 10^5$ CFU/kg feed or $5 \times 10^8/5 \times 10^6$ CFU/kg feed (the recommended dose). Study 3 included three Lavipan® diets supplemented with the additive to levels of LAB/*S. cerevisiae* of: $5 \times 10^8/5 \times 10^6$ CFU/kg feed (the recommended dose), $5 \times 10^9/5 \times 10^7$ CFU/kg feed or $5.5 \times 10^{10}/5.5 \times 10^8$ CFU/kg feed (Table 1). In all cases, doses were confirmed by analysis of feed.³⁹ In study 4, there were three Lavipan® diets containing the additive at the intended doses of 5×10^7 , 5×10^8 or 5×10^9 CFU/kg. Although, no differential counts were specified, it is assumed that they refer to total LAB. Doses were not confirmed by analysis. Birds were weighed on a weekly basis on the first three studies and at days 14 and 41 in study 4. Feed intake, morbidity and mortality were monitored and feed to gain ratio calculated. However, the feed intake data were not reported in the first three studies. Data were analysed using ANOVA and Duncan's test using the pen as experimental unit. Results are presented in Table 1.

Table 1: Summary of performance data of chickens receiving Lavipan®

Trial no	Duration of the trial (days)	Total number of animals		Additive CFU (LAB/ <i>S. cerevisiae</i>)/ kg feed ⁽¹⁾	Feed intake (kg)	Final weight (kg)	Feed: gain	Mortality (%)
		No of replicates per treatment	No of animals per replicate					
1	42	400		0	n.r.	2.66 ^a	1.74	1.5
		4 x 50		$5 \times 10^8/5 \times 10^6$	n.r.	2.76 ^b	1.71	1.5
2	41	750		0	n.r.	2.70 ^a	1.59	1.2
		5 x 50		$5 \times 10^7/5 \times 10^5$	n.r.	2.82 ^b	1.57	0.4
				$5 \times 10^8/5 \times 10^6$	n.r.	2.84 ^b	1.56	1.2

³⁵ Technical dossier/Section IV/Annex IV.2.

³⁶ Technical dossier/Supplementary information May 2015/Annex 13/Annex 13.I.

³⁷ Technical dossier/Supplementary information November 2015/Annex 7.

³⁸ Technical dossier/Supplementary information May 2015/Annex 13/Annex 13.II.

³⁹ Technical dossier/Supplementary information November 2015/Annex 6.

Trial no	Duration of the trial (days)	Total number of animals		Additive CFU (LAB/ <i>S. cerevisiae</i>)/ kg feed ⁽¹⁾	Feed intake (kg)	Final weight (kg)	Feed: gain	Mortality (%)
		No of replicates per treatment × No of animals per replicate						
3	35	160		0	n.r.	2.18 ^a	1.59 ^a	5
		20 × 2		$5 \times 10^8/5 \times 10^6$	n.r.	2.35 ^b	1.51 ^b	5
				$5 \times 10^9/5 \times 10^7$	n.r.	2.38 ^b	1.50 ^b	5
				$5.5 \times 10^{10}/5.5 \times 10^8$	n.r.	2.29 ^b	1.50 ^b	2.5
4	41	256		0	4.1	2.51	1.64	3
		8 × 8		5×10^7	4.0	2.43	1.62	3
				5×10^8	4.1	2.51	1.63	0
				5×10^9	4.1	2.51	1.65	2

n.r. not reported; CFU: colony forming unit; LAB: lactic acid bacteria.

a, b: Means within a column with different superscript letters are significantly different at $p \leq 0.05$.

(1): In study 4, no differential values specified.

Mortality was not influenced by treatment in any of the studies. Birds from the Lavipan® group showed a significantly greater body weight than those in the control group in the first three studies. Feed to gain ratio was also significantly improved in study 3 (feed intake data not given). No parameter was influenced by treatment in study 4. In addition, in this study, use of a single value for CFU does not relate to the specifications provided for the additive.

The FEEDAP Panel notes that in study 3 chickens were kept in cages (two birds per cage, size of cages was not reported). Although this does not reflect current European production practices, the nature of the housing is unlikely to have affected the biological response to the additive.

3.3.2.1. Conclusions on efficacy for chickens for fattening

The FEEDAP Panel concludes that Lavipan® at the recommended dose (5×10^8 CFU LAB/kg feed and 5×10^6 CFU *S. cerevisiae*/kg feed) has the potential to increase final body weight of chickens for fattening.

3.3.3. Efficacy for turkeys for fattening

Four studies with turkeys for fattening conducted in the same Member State but in three different locations were submitted. However, two studies were not considered, one study⁴⁰ due to the lack of replications and the other⁴¹ due to incomplete reporting of data and because birds entered the study at 3 weeks of age.

The first of the remaining studies involved 300 turkeys for fattening (BIG 6, females, 1-day-old) distributed into three treatment groups, each replicated five times.⁴² The diets of the treatment groups included no additive (control) or Lavipan® to levels of LAB/*S. cerevisiae* of $5 \times 10^7/5 \times 10^5$ CFU/kg feed or $5 \times 10^8/5 \times 10^6$ CFU/kg feed (the recommended dose). Doses were confirmed by analysis of feed.⁴³ Birds were fed *ad libitum* the diets in mash form based on wheat/corn/soybean meal. All diets contained a coccidiostat (diclazuril). The trial lasted 105 days. Body weight of birds was measured on a weekly basis. Feed intake and mortality were also monitored and feed to gain calculated. However, data on feed intake were not provided. Data were analysed using one-way ANOVA and Duncan's test considering the pen as experimental unit.

Mortality was low (control = 5, Lavipan® = 4 and 5) and not treatment related. The turkeys fed with Lavipan® at both doses showed a significantly greater final body weight than those of the control group (9.27 vs 9.53 and 9.57 kg, $p < 0.05$). No significant effect on feed to gain ratio was observed. No raw data were provided for the feed intake.

⁴⁰ Technical dossier/Section IV and Supplementary information May 2015/Annexes IV.3 and 14 i 15/Annex 1415I.

⁴¹ Technical dossier/Supplementary information May 2015 and April 2016/Annex 14 i 15/Annex 1415II.

⁴² Technical dossier/Supplementary information May 2015/Annex 14 i 15/Annex 1415III.

⁴³ Technical dossier/Supplementary information November 2015/Annex 8.

The second study involved 112 turkeys (Big six, females, 1-day-old) divided into four treatments of four replicates each (seven turkeys/pen).⁴⁴ The treatments were either a negative control in which animals received the basal diet without Lavipan® or three groups in which animals received diets supplemented with Lavipan® to levels of LAB/*S. cerevisiae* of $5 \times 10^8/5 \times 10^6$ CFU/kg (the recommended dose), $5 \times 10^9/5 \times 10^7$ CFU/kg or $5.5 \times 10^{10}/5.5 \times 10^8$ CFU/kg, respectively. All doses were confirmed by analysis of feed. Birds were fed a basal diet based on triticale/wheat/soybean/maize during the 87 days of the trial. Birds were weighed every 2 weeks and feed intake, morbidity and mortality were monitored during the whole experimental period. However, data on feed intake were not provided. Feed to gain ratio was calculated. Data were analysed using ANOVA considering the pen as experimental unit.

Mortality was low (1%) and not treatment related. The turkeys fed with Lavipan® at the recommended dose showed significantly greater final body weights (7.94 vs 8.18 kg, $p < 0.05$) and average daily weight gain (91 vs 93 g, $p < 0.01$) than those of the control group. No significant effect on feed to gain ratio was observed.

3.3.3.1. Conclusions on efficacy for turkeys for fattening

Only two studies showed positive effects of the supplementation with the minimum recommended dose in the performance of turkeys for fattening. Therefore, the FEEDAP Panel was not able to conclude on the efficacy of Lavipan® for turkeys for fattening based on the data available.

3.3.4. Compatibility with coccidiostats

MIC values were determined for the six coccidiostats (diclazuril, salinomycin sodium, decoquinate, maduramicin and narasin+nicarbazine) for each of the bacterial strains present in Lavipan®.⁴⁵ Values were in all cases greater than four times the maximum authorised dose in feed. Therefore, compatibility of *Lc. lactis* B/00039, *C. divergens* KKP 2012p, *Lb. casei* B/00080 and *Lb. plantarum* B/00081 with diclazuril, salinomycin sodium, decoquinate, maduramicin and narasin+nicarbazine is presumed.

3.4. 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 Regulation⁴⁶ and Good Manufacturing Practice.

4. Conclusions

The identity of the strains *Lc. lactis* B/00039, *C. divergens* KKP 2012p, *Lb. casei* B/00080, *Lb. plantarum* B/00081 and *S. cerevisiae* KKP 2059p has been established. No antibiotic resistance of concern has been detected for the bacterial strains. Following the QPS approach to safety assessment, these strains are presumed safe for the target species, consumers of products from animals fed the additive and the environment.

Lavipan® is not toxic by inhalation or a dermal/ocular irritant, but should be considered as a potential respiratory sensitiser. In the absence of data, no conclusion can be drawn on the skin sensitisation potential.

Lavipan® has the potential to improve the performance of chickens for fattening when supplemented at the recommended dose of 5×10^8 CFU LAB/kg feed and 5×10^6 CFU *S. cerevisiae*/kg feed. No conclusions can be drawn on the efficacy of Lavipan® when added to feed for weaned piglets or turkeys for fattening.

The use of Lavipan® is compatible with diclazuril, salinomycin, decoquinate, maduramicin and narasin+nicarbazine.

⁴⁴ Technical dossier/Supplementary information November 2015/Annex 9.

⁴⁵ Technical dossier/Supplementary information November 2015/Annex 10.

⁴⁶ Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Documentation provided to EFSA

- 1) Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) for weaned piglets, chickens for fattening and turkeys for fattening. October 2013. Submitted by JHJ LTD.
- 2) Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) for weaned piglets, chickens for fattening and turkeys for fattening. Supplementary information. May 2015 Year. Submitted by JHJ LTD.
- 3) Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) for weaned piglets, chickens for fattening and turkeys for fattening. Supplementary information. November 2015. Submitted by JHJ LTD.
- 4) Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081 and *Saccharomyces cerevisiae* KKP 2059p) for weaned piglets, chickens for fattening and turkeys for fattening. Supplementary information. April 2016. Submitted by JHJ LTD.
- 5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Lavipan®.
- 6) Comments from Member States.

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- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Guidance on studies concerning the safety of use of the additive for users/workers. EFSA Journal 2012;10(1):2539, 5 pp. doi:10.2903/j.efsa.2012.2539
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. doi:10.2903/j.efsa.2012.2740

Abbreviations

ANOVA	analysis of variance
bw	body weight
CEN	European Committee for Standardization
CFU	colony forming unit
CV	coefficient of variation

EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
ISO	International Organization for Standardization
LAB	lactic acid bacteria
LOD	limit of detection
MIC	minimum inhibitory concentration
OECD	Organisation for Economic Co-operation and Development
PCM	Polish Collection of Microorganisms
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
RH	relative humidity

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Lavipan®¹

In the current application authorisation is sought under Article 4(1) for *Lavipan*® under the category/functional group 4(b) 'zootechnical additives'/ 'gut flora stabilisers', according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorization is sought for the use of the *feed additive* for piglets (weaned), chickens for fattening and turkeys for fattening.

According to the Applicant, the *feed additive* contains five non-genetically modified microorganisms: *Saccharomyces cerevisiae* LOCK 0141, *Lactococcus lactis* IBB500, *Carnobacterium divergens* S1, *Lactobacillus casei* LOCK 0915 and *Lactobacillus plantarum* LOCK 0862. The strain *Lactococcus lactis* IBB500 is deposited at the Polish Collection of Microorganisms (PCM, Wroclaw, Poland), the strain *Carnobacterium divergens* S1 is deposited at the Collection of Industrial Microorganisms (IAFB, Warsaw) while the other three strains are deposited at the Centre of Industrial Microorganisms Collection (LOCK, Lodz, Poland).

The product is intended to be marketed as a light cream to light brown powder containing at least 1×10^5 colony forming units (CFU) *Saccharomyces cerevisiae* LOCK 0141 per gram and a minimum of 1×10^7 CFU bacterial active substances per gram. The feed additive is intended to be used in feedingstuffs at a minimum dose of 5×10^6 CFU of *Saccharomyces cerevisiae* LOCK 0141 per kg and 5×10^8 CFU of bacterial active substances per kg.

The Applicant used 16S rRNA gene sequence analysis for the identification and characterisation of the bacterial strains and internal transcribed spacer rRNA gene sequence analysis for the identification and characterisation of *Saccharomyces cerevisiae* LOCK 0141. In addition, the Applicant referred to Pulsed Field Gel Electrophoresis (PFGE), a generally recognised standard methodology for the genetic identification of the bacterial strains and to Polymerase Chain Reaction (PCR) for the identification of *Saccharomyces cerevisiae* LOCK 0141. The European Union Reference Laboratory (EURL) recommends for official control these two methods (PFGE and PCR) for the identification of the bacterial and yeast strains.

For the enumeration of *Lactococcus lactis* IBB500, *Carnobacterium divergens* S1, *Lactobacillus casei* LOCK 0915 and *Lactobacillus plantarum* LOCK 0862 in the feed additive and feedingstuffs the Applicant submitted the ISO 15214 poured-plate method and demonstrated its suitability by providing experimental data obtained in the frame of the stability study. The EURL identified instead, for the enumeration of the Lactobacilli in the feed additive and feedingstuffs, the ring-trial validated spread plate method EN 15787 for the enumeration in the feed additive and feedingstuffs.

For the enumeration of *Saccharomyces cerevisiae* LOCK 0141 in feed additive and feedingstuffs the Applicant submitted the EN 15789 poured-plate method.

Based on the performance characteristics available the EURL recommends for official control the five ring trial validated CEN and ISO methods mentioned above.

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.

¹ The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2013-0048_Lavipan®_online.pdf