SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 27 June 2017

doi: 10.2903/j.efsa.2017.4917

Lactobacillus fermentum CECT 5716 and a reduction of the *Staphylococcus* load in breast milk which reduces the risk of infectious mastitis: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Martin Stern, Daniel Tomé, Henk Van Loveren, Marco Vinceti, Peter Willatts, Ambroise Martin, John Joseph Strain and Alfonso Siani

Abstract

Following an application from Biosearch Life, submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus fermentum* CECT 5716 and decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for infectious mastitis. The scope of the application was proposed to fall under a health claim referring to disease risk reduction. The Panel considers that *Lactobacillus fermentum* CECT 5716 is sufficiently characterised. In the context of this application, the *Staphylococcus* load in breast milk can be considered a risk factor for the development of infectious mastitis, as long as evidence is provided that the consumption of *Lactobacillus fermentum* CECT 5716 reduces the *Staphylococcus* load in breast milk as well as the incidence of infectious mastitis. Three human intervention studies investigated the effect of *Lactobacillus fermentum* CECT 5716 on the *Staphylococcus* load of breast milk in lactating women. One of these studies was conducted in lactating women free of infectious mastitis at baseline. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim owing to important methodological limitations. The other two studies were conducted in lactating women with infectious mastitis and, therefore, the effect of the intervention on the incidence of infectious mastitis cannot be assessed. The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus fermentum* CECT 5716 and a reduction of the *Staphylococcus* load in breast milk which reduces the risk of infectious mastitis.

Keywords: *Lactobacillus fermentum* CECT 5716, *Staphylococcus*, breast milk, infectious mastitis, health claim

Requestor: Competent Authority of France following an application by Biosearch Life

Question number: EFSA-Q-2016-00318

Correspondence: nda@efsa.europa.eu

Panel members: Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry J. McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Martin Stern, Daniel Tomé, Dominique Turck, Hendrik Van Loveren, Marco Vinceti and Peter Willatts.

Competing interests: In line with EFSA's policy on declarations of interest, Panel member Anders Sjödin did not participate in the development and adoption of this scientific opinion.

Acknowledgements: The Panel wishes to thank the members of the Working Group on Claims: Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Harry J. McArdle, Yolanda Sanz, Alfonso Siani, Sean (J.J.) Strain, Hendrik Van Loveren and Peter Willatts, for the preparatory work on this scientific output.

Suggested citation: EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst K-I, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Stern M, Tomé D, Van Loveren H, Vinceti M, Willatts P, Martin A, Strain JJ and Siani A, 2017. Scientific Opinion on the *Lactobacillus fermentum* CECT 5716 and a reduction of the *Staphylococcus* load in breast milk which reduces the risk of infectious mastitis: evaluation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006. EFSA Journal 2017;15(7):4917, 13 pp. https://doi.org/10.2903/j.efsa.2017.4917

ISSN: 1831-4732

Summary

Following an application from Biosearch Life, submitted for authorisation of a health claim pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of France, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus fermentum* CECT 5716 and decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for infectious mastitis.

The scope of the application was proposed to fall under a health claim referring to disease risk reduction.

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA general guidance for stakeholders on health claim applications and the EFSA guidance on the scientific requirements for health claims related to gut and immune function.

The food/constituent which is the subject of the health claim is *Lactobacillus fermentum* CECT 5716. The Panel considers that *Lactobacillus fermentum* CECT 5716 is sufficiently characterised.

The claimed effect proposed by the applicant is 'decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for mammary bacterial dysbiosis/mastitis'. The target population proposed by the applicant is the general population of lactating women.

The Panel considers that, although a high *Staphylococcus* load in breast milk is associated with an increased risk of developing infectious mastitis, no evidence has been provided to establish that a decrease in the *Staphylococcus* load of breast milk generally reduces the risk of developing infectious mastitis. In the context of this application, the *Staphylococcus* load of breast milk can be considered as a risk factor for the development of infectious mastitis, as long as evidence is provided that the consumption of the food constituent which is the subject of the health claim reduces the *Staphylococcus* load in breast milk as well as the incidence of infectious mastitis.

Three human intervention studies investigated the effect of consuming *Lactobacillus fermentum* CECT 5716 on the *Staphylococcus* load of breast milk in lactating women.

One of these studies was conducted in lactating women free of infectious mastitis at baseline, and the effect of *Lactobacillus fermentum* CECT 5716 on the incidence of infectious mastitis was also assessed. The Panel considers that, owing to important methodological limitations (e.g. inappropriate randomisation, risk of selection bias, statistical analysis for completers only, multicentre design of the study not considered in data analysis, no correction for multiple secondary outcomes) and high (> 50%) dropout rate which may introduce selection bias, no conclusions can be drawn from this study for the scientific substantiation of the claim.

The Panel notes that the two other studies which investigated the effect of consuming *Lactobacillus fermentum* CECT 5716 on the *Staphylococcus* load in breast milk were conducted in lactating women with infectious mastitis at baseline and that they do not allow investigating the effect of consuming *Lactobacillus fermentum* CECT 5716 on the risk of developing infectious mastitis.

The applicant also provided a number of studies in support of the mechanisms by which *Lactobacillus fermentum* CECT 5716 could exert the claimed effect.

The Panel considers that, in the absence of evidence for an effect on the incidence of infectious mastitis *in vivo* in humans, the results of human studies investigating the effects of *Lactobacillus fermentum* CECT 5716 on the proposed risk factor (*Staphylococcus* load) only, and the studies on the mechanisms by which the food/constituent could exert the claimed effect, cannot be used as a source of data for the scientific substantiation of the claim.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus fermentum* CECT 5716 and a reduction of the *Staphylococcus* load in breast milk which reduces the risk of infectious mastitis.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006[1] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14–17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to of this Regulation, an application for shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: *Lactobacillus fermentum* CECT 5716 and decreases the *Staphylococcus* load in breast milk.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of *Lactobacillus fermentum* CECT 5716, a positive assessment of its safety, nor a decision on whether *Lactobacillus fermentum* CECT 5716 is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food constituent that is the subject of the claim is *Lactobacillus fermentum* CECT 5716, a bacterial strain originally isolated from human breast milk.

Health relationship as claimed by the applicant

According to the applicant, the constituent (*Lactobacillus fermentum* CECT 5716) 'decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for mammary bacterial dysbiosis/mastitis'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

According to the applicant, the effect of *Lactobacillus fermentum* CECT 5716 could potentially be explained by exerting either localised antimicrobial, and/or anti-inflammatory effects in the breast tissue.

According to the applicant, 'although further studies are required to elucidate the pathways that lactobacilli may follow to colonise the mammary gland after oral ingestion, evidences involve immune cells in this transport. Also it was detected a general association of *Staphylococcus* with IL-8. This cytokine (IL-8) elicits the infiltration of immune cells to the site of infection, and its concentration in human milk has been proposed as an effective indicator of mastitis'.

'The relatively low counts of *Lactobacillus* vs *Staphylococcus* in the breast milk samples suggest that the mechanism for the decreased pain is unlikely to be simply a "competition phenomenon"'.

[1] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

'In addition to the probiotic effect on intestinal function, *Lactobacillus fermentum* CECT 5716 also shows immunomodulatory or antimicrobial effects in human, in animal and *in vitro*. *Lactobacillus fermentum* CECT 5716 has been found to inhibit the growth of a wide spectrum of pathogenic bacteria, has a role in supporting maturation of the infant immune system by acting on both innate and acquired immunity through a variety of mechanisms, has an anti-inflammatory activity and presents an *in vivo* efficacy to reduce the incidence of gastrointestinal and upper respiratory tract infections in infants'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: '*Lactobacillus fermentum* CECT 5716 decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for mammary bacterial dysbiosis/mastitis'.

Specific conditions of use as proposed by the applicant

The applicant has proposed an intake of 3×10^9 CFU/day of *Lactobacillus fermentum* CECT 5716, as a capsule. The target population proposed by the applicant is the general population of lactating women.

Data provided by the applicant

Health claim application on *Lactobacillus fermentum* CECT 5716 decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for mammary bacterial dysbiosis/mastitis pursuant to Article 14 of Regulation 1924/2006, presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims.[2]

As outlined in the General guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a), it is the responsibility of the applicant to provide the totality of the available evidence.

This health claim application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006. Data claimed to be proprietary and confidential by the applicant include:

Data related to the unpublished human study (Hurtado, unpublished) are proprietary to Biosearch Life.

Data related to the manufacturing process of *Lactobacillus fermentum* CECT 5716 presented in the application are confidential to Biosearch Life.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claims applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016a).

The scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms are outlined in a specific EFSA guidance (EFSA NDA Panel, 2016b).

3. Assessment

3.1. Characterisation of the food/constituent

The food/constituent that is the subject of the health claim is *Lactobacillus fermentum* CECT 5716. The species and strain identity and the characteristics of *L. fermentum* CECT 5716 have been determined using phenotypic and genotypic methods as indicated in the references provided (Martín et al., 2003, 2005; Xaus et al., 2003). The whole genome of *L. fermentum* CECT 5716 has been sequenced (Jiménez et al., 2010).

A culture collection number from the Spanish Type Culture Collection (CECT) is indicated. CECT accepts deposits as a restricted-access non-public International Depositary Authority under the Budapest Treaty.

The Panel considers that the food/constituent *Lactobacillus fermentum* CECT 5716, which is the subject of the health claim, is sufficiently characterised.

[2] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific and technical guidance for the preparation and presentation of an application for authorisation of a health claim (revision 1). EFSA Journal 2011;9(5):2170. [36 pp.]. https://doi.org/10.2903/j.efsa.2011.2170

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for mammary bacterial dysbiosis/mastitis'. The target population proposed by the applicant is the general population of lactating women.

Mastitis is defined as an inflammatory condition of the breast. The two main causes of mastitis are milk stasis and infection. Milk stasis is usually the primary cause, which may or may not be accompanied by (or progress to) infection (WHO, 2000).

Upon a request from EFSA, the applicant specified that the disease to which the risk factor relates is lactational infectious mastitis and that the risk factor proposed is *Staphylococcus* load in breast milk.

The Panel notes that, on the basis of leukocytes and bacteria counts in milk from breasts with clinical signs of mastitis, the following classification has been proposed (WHO, 2000):

- Milk stasis: bacteria $< 10^3$ CFU/mL milk and leukocytes $< 10^6$/mL milk.
- Non-infectious mastitis: bacteria $< 10^3$ CFU/mL milk and leukocytes $> 10^6$/mL milk.
- Infectious mastitis: bacteria $> 10^3$ CFU/mL milk and leukocytes $> 10^6$/mL milk.

While milk stasis tends to improve with breast-feeding alone, non-infectious mastitis requires additional expression of milk after a feed. Without effective removal of milk, non-infectious mastitis is likely to progress to infectious mastitis. Infectious mastitis is treated with both transfer of milk to the breastfed infant and systemic antibiotics.

The Panel considers that, whereas mastitis is generally diagnosed in the clinical setting by the presence of local symptoms (e.g. breast pain, redness, lump), with or without systemic symptoms (e.g. fever, shivering, hot sweats or aches), the diagnosis of infectious mastitis needs to be confirmed by microbiological analysis of breast milk. The Panel notes that recent studies assessing the bacterial load of breast milk from women with no clinical signs or symptoms of mastitis show bacterial counts in breast milk $> 10^3$ CFU/mL milk (Obermajer et al., 2015; Cabrera-Rubio et al., 2016) . The Panel also notes that the threshold of bacterial load in breast milk proposed by WHO for the diagnosis of infectious mastitis was established using cultural methods. Genetic methods quantify the presence of bacterial DNA even when bacteria are not able to grow in culture medium, and therefore, the measurements of the bacterial load obtained with these techniques are often higher than those obtained using cultural methods. Therefore, the Panel considers that measures of bacterial load in breast milk may vary depending on the methodology used for analysis, and that the same methodology should be used within a study for between-group comparisons of the bacterial load in breast milk.

Upon a request from EFSA for clarification on whether the risk factor proposed is the *Staphylococcus* load or rather the load of specific species of the genus *Staphylococcus*, the applicant clarified that *Staphylococcus aureus* is the main aetiological factor of acute mastitis (Contreras and Rodríguez, 2011; Fernandez et al., 2014; LaTuga et al., 2014), while overgrowth of *Staphylococcus epidermidis* is observed predominantly in patients with subacute mastitis (Jiménez et al., 2015).

Upon a request from EFSA to provide evidence that there is an independent association between the proposed risk factor (*Staphylococcus* load) and the proposed disease (infectious mastitis), the applicant submitted a publication showing that the presence of *S. aureus* in the nipple and in breast milk increases the risk of developing infectious mastitis. In a longitudinal study from birth to weeks 1–8 post-partum, Cullinane et al. (2015) reported that women with *S. aureus* isolated from their nipple or milk at week 1 of the study had an increased risk of subsequently developing infectious mastitis (with incidence rate (IR) 1.72, 95% CI 1.04–2.85 and 1.78, 95% CI 1.08–2.92, respectively).

Upon a request from EFSA to provide evidence that a modification of the risk factor prospectively modifies the risk of the disease, the applicant referred to the three human studies submitted for the substantiation of the claim. The Panel notes that only one of these three studies has investigated the development of the disease prospectively (Hurtado, 2016; unpublished).

The Panel considers that, although a high *Staphylococcus* load in breast milk is associated with an increased risk of developing infectious mastitis, no evidence has been provided to establish that a decrease in the *Staphylococcus* load of breast milk generally reduces the risk of developing infectious mastitis. In the context of this application, the *Staphylococcus* load of breast milk can be considered as a risk factor for the development of infectious mastitis, as long as evidence is provided that the consumption of the food constituent which is the subject of the health claim reduces the *Staphylococcus* load in breast milk as well as the incidence of infectious mastitis.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed and Google scholar with the following key words: *Lactobacillus fermentum,* CECT 5716*, Lactobacillus fermentum* CECT 5716, lactation, breast, immune, mastitis. No restrictions were applied. Hand searches were also performed.

The applicant provided three human intervention studies as being pertinent to the claim.

Three human intervention studies investigated the effect of consuming *Lactobacillus fermentum* CECT 5716 on the *Staphylococcus* load of breast milk in lactating women (Arroyo et al., 2010; Maldonado-Lobón et al., 2015; Hurtado, 2016; unpublished). One of these studies (Hurtado, 2016; unpublished, claimed as proprietary by the applicant) was conducted in lactating women free of infectious mastitis at baseline, and the effect of *Lactobacillus fermentum* CECT 5716 on the incidence of infectious mastitis was also assessed. The remaining two studies were conducted in lactating women with a diagnosis of infectious mastitis at baseline (Arroyo et al., 2010; Maldonado-Lobón et al., 2015).

Upon a request from EFSA, the applicant clarified that, in all three studies, the diagnosis of mastitis required a clinical diagnosis by a physician (i.e. based on the presence of at least two out of three local symptoms in the breast (pain, redness, lump) and at least one systemic symptom (shivering, hot sweats or aches), and microbiological confirmation in breast milk (total bacteria in agar plates $> 10^3$ CFU/mL). The applicant also clarified that the method used for the collection of breast milk samples was similar in all human studies submitted (cleaning of the nipple and mammary areola followed by the use of an antibacterial solution). The breast milk sample was collected by manual expression in a sterile tube. The first drops of milk were discarded to avoid contamination by the antibacterial solution used for disinfection (chlorhexidine).

In a randomised, parallel, two-arm, double-blind, placebo-controlled multicentre study (12 centres) (Hurtado, 2016, unpublished, claimed as proprietary by the applicant), the effect of *Lactobacillus fermentum* CECT 5716 on the incidence of infectious mastitis was assessed in healthy lactating women during the first 4 months of lactation.

Women 18–45 years intending to breast-feed, with delivery between 37 and 42 weeks of gestation, who had received at least one dose of preventive antibiotic treatment between 48 h before and 48 h after child birth were recruited 1–6 days after delivery. Mothers and children with pathologies that could hinder or preclude breastfeeding were excluded. Upon a request from EFSA to clarify why antibiotic treatment was used as an inclusion criterion, the applicant explained that it is estimated that > 40% of pregnant women are given some type of antibiotic therapy immediately prior to delivery (Ledger and Blaser, 2013), and that women receiving antibiotic therapy around delivery have higher risk of developing infectious mastitis (Odds Ratio 1.53; Mediano et al., 2014). Therefore, only women receiving antibiotic therapy were included in the study in order to avoid the possible interaction of this variable with the occurrence of infectious mastitis.

The incidence of infectious mastitis was the main outcome of the study. The diagnosis of mastitis was made by a clinician and required the presence of at least two out of three local symptoms in the breast (pain, redness, lump) and at least one systemic symptom (shivering, hot sweats or aches), as well as microbiological confirmation (total bacteria in agar plates $> 10^3$ CFU/mL). Secondary outcomes were microbiota and markers of inflammation (interleukin-8 (IL-8)) in breast milk at baseline and at the end of the intervention and in mastitis events, breast pain, the assessment of microbiota in infant faeces at the end of the study and general health and growth of infants.

Bacteriological assessments included total aerobic and anaerobic bacteria by plate counting and specific bacterial genera by real-time PCR (including *Staphylococcus*, *Lactobacillus* and *Streptococcus*), as explained by the applicant upon a request by EFSA. For breast pain evaluation, a subjective numerical scale ranging from 1 (no pain) to 10 (extremely painful) was used monthly.

Upon a request from EFSA for clarification, the applicant explained that the study visits were scheduled at the beginning and end of the study. In addition, participants received three phone calls (one per month), and additional visits were scheduled in case of "medical problems related to the breast".

Power calculations assumed an incidence rate of infectious mastitis of 0.17 and a 40% reduction in the intervention group, with a power of 80% and $\alpha = 0.05$. It was estimated that a total of 516 subjects (258 per group) would be needed.

Upon a request from EFSA for clarification, the applicant explained that a competitive recruitment was carried out in the 12 centres, with no limit on the number of participants to be recruited on each centre. Two centres decided not to participate in the study 'because of internal hospital issues'. A

randomisation list containing 1,000 numbers was generated by computer program. Each centre received 50 numbers. Additional numbers were distributed to centres recruiting more participants.

A total of 625 women were recruited in the 12 centres combined (918 subject codes distributed among the centres) and were randomised to consume one capsule daily of either *Lactobacillus fermentum* CECT 5716 (3×10^9 CFU, n = 303) or placebo (maltodextrin, n = 322) for 16 weeks.

No significant differences in baseline characteristics between the test and control groups were reported for either the women (e.g. level of education, living area, contact with animals, previous mastitis, previous parity, gestational days, type of birth, etc.) or the infants (e.g. anthropometry, infections, number of hours until first contact with the breast, use of formula feeding, difficulty in sucking during breastfeeding, duration of breastfeeding, etc.), except for the use of anaesthesia at birth, which was significantly higher in the test group.

All participants obtained the same breastfeeding recommendations, compatible with the recommendations from WHO[3] and the Spanish Association of Paediatrics.[4] In case of mastitis symptoms, clinical diagnosis was made by the corresponding physician. All women with symptoms received recommendations on how to improve their infant's attachment to the breast and advice to breastfeed frequently to improve milk transfer. Pharmacological treatment was prescribed based on the severity and duration of symptoms. Analgesics were prescribed in case of pain and antibiotics in case of fever > 38°C or duration of severe symptoms for more than 24–48 h. The Panel notes that the definition of 'severe' symptoms was not given and the type of the recommended antibiotic(s) and the recommended duration of antibiotic treatment were not specified.

A total of 291 women (139 in the test group and 152 in the control group) completed the study. The reasons for dropouts of 334 women (53.4% of the women randomised), 164 in the treatment group (54%) and 170 in the placebo group (52.8%) were reported. Main reasons for withdrawal were lost to follow-up (33 women per group), stop of breastfeeding because of perception of insufficient milk volume, stop of breastfeeding because of mother's decision, voluntary resignation. The Panel notes that more than 50% of the women randomised dropped from the study. The statistical analysis of the results was presented for completers only.

The authors retrospectively recalculated the power of the study using the final results. Owing to an overall incidence rate higher than expected, and to a higher reduction on the incidence of mastitis in the test group compared to controls than initially estimated, the applicant claims that the statistical power of the study was kept, although the number of women who completed the intervention was lower than initially calculated.

Upon a request from EFSA, the applicant indicated that the main outcome of the study (incidence of infectious mastitis) could only be analysed in the group of women who completed the study and that no imputation for missing data was applied at any time of the study. The applicant also indicated that for bacterial load and IL-8, milk samples were collected at baseline and at the end of intervention and data of all subjects included in the study (not only of those who completed the study) were taken into account for comparison of microbiological endpoints at these two time points. The number of dropouts was similarly distributed among the two study groups. The applicant provided comparisons of the baseline characteristics between dropouts and completers. Significant differences in baseline characteristics were observed between women who completed the study and those who dropped out. Women who completed the study were significantly older, with a higher level of education, less likely to smoke, less likely to use feeding bottle, and their infants were older at the time of recruitment as compared to women who dropped out. It was also reported that the two groups differed regarding the use of pacifier by infants, being higher in group of dropouts, and on the number of siblings, being higher in the group of completers. The applicant claims that none of these variables was associated with mastitis events and that the differences observed between dropouts and completers could not impact the primary outcome of the study. The Panel notes, however, that the information provided by the applicant does not allow establishing whether the differences between completers and dropouts regarding these baseline characteristics would have invalidated randomisation and introduced selection bias into the analysis.

Incidence rates of infectious mastitis were calculated for each group. A grouping variable considering 0 events vs 1 or more events along study period was also considered and odds ratios were calculated. A Poisson regression model was applied to adjust the number of events by relevant covariates. A step-wise regression model (forward method) was used to determine the associated

[3] http://www.who.int/maternal_child_adolescent/topics/child/nutrition/breastfeeding/en/
[4] http://www.aeped.es

covariates to be included in the final model. Only the number of previous pregnancies was found to be significantly associated with the incidence of infectious mastitis and was considered as covariate in the final model. Bacterial loads were analysed using paired t-test or non-parametric related samples tests, depending on whether variables were normally distributed or not. Logistic regression models were applied to mastitis incidence in order to assess the association between bacterial loads and concentrations of IL-8 in breast milk with events of infectious mastitis. Information on the use of correction for multiple testing for secondary outcomes was not provided.

Upon a request from EFSA, the number of subjects randomised in each centre and the results for the primary outcome by centre were provided by the applicant. The Panel notes that the number of subjects recruited in each centre varied between one subject (in two centres, 0.3% of the total sample) and 86 subjects (29.6% of the total sample), with five centres recruiting seven subjects or less and three centres recruiting zero participants in either the test or the control group. The Panel also notes that randomisation was not stratified by centre and that the multicentre design of the study was not considered in data analysis.

The total number of infectious mastitis during the study was 58 (18 in the test group and 40 in the placebo group). A total of nine women had recurrent events of infectious mastitis, two in the test group (two events each) and seven in the placebo group (four had two events and three had three events). The applicant claims that a similar distribution of events was observed in all centres. The Panel notes, however, that even excluding the five centres with seven subjects or less, the % of women with at least one event of mastitis ranged between 0% and 20% in the test group and between 8.6% and 57% in the control group, depending on the centre.

Upon a request from EFSA regarding the procedure followed for the treatment of infectious mastitis, the applicant indicated that eight events of infectious mastitis (out of 18) were treated with analgesics and eight with antibiotics in the intervention group. In the control group, 18 events (out of 40) were treated with analgesics and eight with antibiotics. These differences were reported not to be statistically significant.

The incidence of particular symptoms related to mastitis was reported, including breast pain, breast nodules, heat zones, red sore areas, mammary tumours, fever, discomfort and sweating. The Panel notes that the number of comparisons made to assess differences in breast symptoms between groups was high (eight symptoms analysed per month and during the whole study period), and that adjustments for multiple comparisons were not made. The Panel considers that these analyses are at high risk of bias.

The Panel considers that, owing to important methodological limitations (e.g. inappropriate randomisation, statistical analysis for completers only, multicentre design of the study not considered in data analysis) and high (> 50%) dropout rate which may introduce selection bias, no conclusions can be drawn from this study for the scientific substantiation of the claim.

The Panel notes that the two other studies which investigated the effect of consuming *Lactobacillus fermentum* CECT 5716 on the *Staphylococcus* load of breast milk (Arroyo et al., 2010; Maldonado-Lobón et al., 2015) were conducted in lactating women with infectious mastitis at baseline and that they do not allow investigating the effect of consuming *Lactobacillus fermentum* CECT 5716 on the risk of developing infectious mastitis.

The applicant also provided a number of studies in support of mechanisms by which *Lactobacillus fermentum* CECT 5716 could exert the claimed effect (Martín et al., 2005; Olivares et al., 2006, 2007; Diaz-Ropero et al., 2007; Arribas et al., 2009; Mañé et al., 2009; Peran et al., 2006, 2007; Pérez-Cano et al., 2010; Gil-Campos et al., 2012; Maldonado et al., 2012; Rodríguez-Nogales et al., 2015).

The Panel considers that, in the absence of evidence for an effect on the incidence of infectious mastitis *in vivo* in humans, the results of human studies investigating the effects of *Lactobacillus fermentum* CECT 5716 on the proposed risk factor (*Staphylococcus* load) only, and the studies provided on the mechanisms by which the food/constituent could exert the claimed effect, cannot be used as a source of data for the scientific substantiation of the claim.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus fermentum* CECT 5716 and a reduction of the *Staphylococcus* load in breast milk which reduces the risk of infectious mastitis.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food/constituent, *Lactobacillus fermentum* CECT 5716, which is the subject of the health claim, is sufficiently characterised.

- The claimed effect proposed by the applicant is 'decreases the *Staphylococcus* load in breast milk. High *Staphylococcus* load in breast milk is a risk factor for mammary bacterial dysbiosis/mastitis'. The target population proposed by the applicant is the general population of lactating women. In the context of this application, the *Staphylococcus* load of breast milk can be considered as a risk factor for the development of infectious mastitis, as long as evidence is provided that the consumption of the food constituent which is the subject of the health claim reduces the *Staphylococcus* load in breast milk as well as the incidence of infectious mastitis.
- A cause and effect relationship has not been established between the consumption of *Lactobacillus fermentum* CECT 5716 and a reduction of the *Staphylococcus* load in breast milk which reduces the risk of infectious mastitis.

Steps taken by EFSA

1) Health claim application on *L. fermentum* CECT 5716 and decrease of *Staphylococcus* load in breast milk pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0447_FR). Submitted by Biosearch Life, Camino de Purchil, 66. 18004 Granada, Spain.
2) This application was received by EFSA on 27/4/2016.
3) The scope of the application was proposed to fall under a health claim referring to disease risk reduction. The application included a request for the protection of proprietary data.
4) The scientific evaluation procedure started on 13/6/2016.
5) On 6/9/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 4/10/2016 and was restarted on 28/10/2016, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
6) On 28/10/2016, EFSA received the applicant's reply (which was made available to EFSA in electronic format on 28/10/2016).
7) On 16/11/2016, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 8/12/2016 and was restarted on 24/2/2017, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
8) On 24/2/2017, EFSA received the applicant's reply (which was made available to EFSA in electronic format on 24/02/2017).
9) On 22/3/2017, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 12/4/2016 and was restarted on 4/5/2017, in compliance with Article 16(1) of Regulation (EC) No 1924/2006.
10) On 4/5/2017, EFSA received the applicant's reply (which was made available to EFSA in electronic format on 4/5/2017).
11) During its meeting on 27/6/2017, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to *L. fermentum* CECT 5716 and decrease of *Staphylococcus* load in milk.

References

Arribas B, Rodríguez-Cabezas ME, Comalada M, Bailón E, Camuesco D, Olivares M, Xaus J, Zarzuelo A and Gálvez J, 2009. Evaluation of the preventative effects exerted by *Lactobacillus fermentum* in an experimental model of septic shock induced in mice. British Journal of Nutrition, 101, 51–58.

Arroyo R, Martín V, Maldonado A, Jiménez E, Fernández L and Rodríguez JM, 2010. Treatment of infectious mastitis during lactation: antibiotics versus oral administration of Lactobacilli isolated from breast milk. Clinical Infectious Disease, 50, 1551–1558.

Cabrera-Rubio R, Mira-Pascual L, Mira A and Collado MC, 2016. Impact of mode of delivery on the milk microbiota composition of healthy women. Journal of Developmental Origin of Health and Disease, 7, 54–60.

Contreras GA and Rodríguez JM, 2011. Mastitis: comparative etiology and epidemiology. Journal of Mammary Gland Biology and Neoplasia, 16, 339–356.

Cullinane M, Amir LH, Donath SM, Garland SM, Tabrizi SN, Payne MS and Bennett CM, 2015. Determinants of mastitis in women in the CASTLE study: a cohort study. BMC Family Practice, 16, 181.

Diaz-Ropero MP, Martín R, Sierra S, Lara-Villoslada F, Rodríguez JM, Xaus J and Olivares M, 2007. Two *Lactobacillus* strains, isolated from breast milk, differently modulate the immune response. Journal of Applied Microbiology, 102, 337–343.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016a. General scientific guidance for stakeholders on health claim applications. EFSA Journal 2016;14(1):4367, 38 pp. https://doi.org/10.2903/j.efsa.2016.4367

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016b. Guidance on the scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms. EFSA Journal 2016;14(1):4369, 23 pp. https://doi.org/10.2903/j.efsa.2016.4369

Fernandez L, Arroyo R, Espinosa I, Marín M, Jiménez E and Rodríguez JM, 2014. Probiotics for human lactational mastitis. Beneficial Microbes, 5, 169–183.

Gil-Campos M, López MÁ, Rodriguez-Benítez MV, Romero J, Roncero I, Linares MD, Maldonado J, López-Huertas E, Berwind R, Ritzenthaler KL, Navas V, Sierra C, Sempere L, Geerlings A, Maldonado-Lobón JA, Valero AD, Lara-Villoslada F and Olivares M, 2012. *Lactobacillus fermentum* CECT 5716 is safe and well tolerated in infants of 1-6 months of age: a randomized controlled trial. Pharmacological Research, 65, 231–238.

Hurtado J, 2016, unpublished study report. Study of nutritional intervention, multicenter, randomized, blind, parallel group to evaluate the effect of the consumption of *Lactobacillus fermentum* CECT5716 Lc40 on mastitis incidence.

Jiménez E, Langa S, Martin V, Arroyo R, Martin R, Fernández L and Rodríguez JM, 2010. Complete genome sequence of *Lactobacillus fermentum* CECT 5716, a probiotic strain isolated from human milk. Journal of Bacteriology, 192, 4800.

Jiménez E, de Andrés J, Manrique M, Pareja-Tobes P, Tobes R, Martínez-Blanch JF, Codoñer FM, Ramón D, Fernández L and Rodríguez JM, 2015. Metagenomic analysis of milk of healthy and mastitis-suffering women. Journal of Human Lactation, 31, 406–415.

LaTuga MS, Stuebe A and Seed PC, 2014. A review of the source and function of microbiota in breast milk. In: Segars JH, Kjersti M (Guest Ed.). *The Microbiome and Reproduction*. Aagaard, Thieme Medical Publishers, Inc, New York, USA. pp. 68–73.

Ledger WJ and Blaser MJ, 2013. Are we using too many antibiotics during pregnancy? A commentary. British Journal of Obstetrics and Gynaecology, 120, 1450–1452.

Maldonado J, Cañabate F, Sempere L, Vela F, Sánchez AR, Narbona E, López-Huertas E, Geerlings A, Valero AD, Olivares M and Lara-Villoslada F, 2012. Human milk probiotic *Lactobacillus fermentum* CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. Journal of Pediatric Gastroenterology and Nutrition, 54, 55–61.

Maldonado-Lobón JA, Díaz-López MA, Carputo R, Duarte P, Díaz-Ropero MP, Valero AD, Sañudo A, Sempere L, Ruiz-López MD, Bañuelos Ó, Fonollá J and Olivares Martín M, 2015. *Lactobacillus fermentum* CECT 5716 reduces Staphylococcus load in the breastmilk of lactating mothers suffering breast pain: a randomized controlled trial. Breastfeeding Medicine, 10, 425–432.

Mañé J, Lorén V, Pedrosa E, Ojanguren I, Xaus J, Cabré E, Domènech E and Gassull MA, 2009. *Lactobacillus fermentum* CECT 5716 prevents and reverts intestinal damage on TNBS-induced colitis in mice. Inflammatory Bowel Diseases, 15, 1155–1163.

Martín R, Langa S, Reviriego C, Jimínez E, Marín ML, Xaus J, Fernández L and Rodríguez JM, 2003. Human milk is a source of lactic acid bacteria for the infant gut. Journal of Pediatrics, 143, 754–758.

Martín R, Olivares M, Marín ML, Fernández L, Xaus J and Rodríguez JM, 2005. Probiotic potential of 3 *Lactobacilli* strains isolated from breast milk. Journal of Human Lactation, 21, 8–17.

Mediano P, Fernandez L, Rodriguez JM and Marin M, 2014. Case-control study of risk factors for infectious mastitis in Spanish breastfeeding women. BMC Pregnancy and Childbirth, 14, 195–208.

Obermajer T, Lipoglavšek L, Tompa G, Treven P, Lorbeg PM, Matijašić BB and Rogelj I, 2015. Colostrum of healthy Slovenian mothers: microbiota composition and bacteriocin gene prevalence. PLoS ONE, 10, e0123324.

Olivares M, Díaz-Ropero MP, Martín R, Rodríguez JM and Xaus J, 2006. Antimicrobial potential of four *Lactobacillus* strains isolated from breast milk. Journal of Applied Microbiology, 101, 72–79.

Olivares M, Díaz-Ropero MP, Sierra S, Lara-Villoslada F, Fonollá J, Navas M, Rodríguez JM and Xaus J, 2007. Oral intake of *Lactobacillus fermentum* CECT5716 enhances the effects of influenza vaccination. Nutrition, 23, 254–260.

Peran L, Camuesco D, Comalada M, Nieto A, Concha A, Adrio JL, Olivares M, Xaus J, Zarzuelo A and Galvez J, 2006. *Lactobacillus fermentum*, a probiotic capable to release glutathione, prevents colonic inflammation in the TNBS model of rat colitis. International Journal of Colorectal Diseases, 21, 737–746.

Peran L, Sierra S, Comalada M, Lara-Villoslada F, Bailón E, Nieto A, Concha A, Olivares M, Zarzuelo A, Xaus J and Gálvez J, 2007. A comparative study of the preventative effects exerted by two probiotics, *Lactobacillus reuteri* and *Lactobacillus fermentum*, in the trinitrobenzenesulfonic acid model of rat colitis. British Journal of Nutrition, 97, 96–103.

Pérez-Cano FJ, Dong H and Yaqoob P, 2010. In vitro immunomodulatory activity of *Lactobacillus fermentum* CECT5716 and *Lactobacillus salivarius* CECT5713: two probiotic strains isolated from human breast milk. Immunobiology, 215, 996–1004.

Rodríguez-Nogales A, Algieri F, Vezza T, Garrido-Mesa N, Olivares M, Comalada M, Riccardi C, Utrilla MP, Rodríguez-Cabezas ME and Galvez J, 2015. The viability of *Lactobacillus fermentum* CECT5716 is not essential to exert intestinal anti-inflammatory properties. Food and Function, 6, 1176–1184.

WHO (World Health Organisation), 2000. Mastitis: causes and management, WHO/FCH/CAH/00.13 Geneva.

Xaus J, Martın R, Olivares M, Langa S, Reviriego C, Boza J, Jimenez J, Marın ML, Fernandez Land Rodrıguez JM, 2003. Human breast milk is a source of probiotics. In: Dietrich Knorr (ed.). *NFIF 2003 New Functional Ingredients and Foods*. Safety, Health and Convenience, EFFoST Meeting, Copenhagen, pp. 9–11, April 2003.

Abbreviations

CECT	Spanish Type Culture Collection
CFU	colony forming units
IL	interleukin
IR	incidence rate
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
PCR	polymerase chain reaction