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SCIENTIFIC OPINION

Lactobacillus plantarum 299v (DSM 9843) and improve iron absorption

Scientific substantiation of a health claim related to *Lactobacillus plantarum* 299v (DSM 9843) and improve iron absorption pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies

(Question No EFSA-Q-2008-785)

Adopted on 13 March 2009

PANEL MEMBERS

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SUMMARY

Following an application from Probi AB submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Sweden, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus plantarum* 299v (DSM 9843) and improve iron absorption.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The food/constituent that is the subject of the claim is "*Lactobacillus plantarum* 299v (DSM 9843)". The bacterial strain *Lactobacillus plantarum* 299v (DSM 9843) is sufficiently characterised.

The claimed effect is to improve iron absorption and the target population is adults at risk for iron deficiency. Iron deficiency is one of the most common micronutrient deficiencies. The Panel considers that improving iron absorption might be beneficial to human health.

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[1] For citation purposes: Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from Probi AB on the scientific substantiation of a health claim related to *Lactobacillus plantarum* 299v (DSM 9843) and improve iron absorption. *The EFSA Journal* (2009) 999, 1-9.

[*] One member of the Panel did not participate in the discussion on the subject referred to above because of possible conflicts of interest.

A total of five publications were identified as relevant by the applicant. Four were human studies which focused on the effect of the strain on non-haem iron absorption from different products. Two of the studies correspond to published data and the other two are unpublished. The fifth study corresponds to an *in vitro* unpublished study.

Two human studies focused on iron absorption from an oat gruel product. The studies were conducted in women at reproductive age with low Fe stores but non-anaemic. One of the studies reports a statistically significant increase on non-haem iron absorption from the test product (fermented oat gruel with the *L. plantarum* 299v) in 24 women. In the second study with 18 women, no effect of the administration of pasteurised fermented oat gruel with added viable lyophilised *L. plantarum* 299v was found.

Another two human studies (unpublished data) focused on iron absorption from an iron supplemented fruit drink in the same target population (women of childbearing age with low Fe stores but non-anaemic). In the first study 10 women consumed a product containing 10^9 cfu *L. plantarum* 299v whilst in the second study conducted in 11 volunteers, the dose was 10^{10} cfu. In the first study iron adsorption from the test product was significantly higher than from the control product whilst in the second study no significant differences were observed.

Weaknesses of the studies, such as short intervention periods, differences in the doses of *L. plantarum* 299v, limited number of subjects, and conflicting findings, were identified by the Panel as factors restricting the studies value in substantiation of the claimed effect.

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of *L. plantarum* 299v and the improvement of iron absorption.

Key words: *Lactobacillus plantarum* 299v (DSM 9843), improve iron absorption

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BACKGROUND

Regulation (EC) No 1924/2006[2] 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 that Regulation and are authorised in accordance with this Regulation and included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of that Regulation lays down provisions for addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence or include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of that Regulation, an application for inclusion in the Community list of permitted claims referred to in Art 13(3) shall be submitted by the applicant to the national competent authority of a Member State, who will make the application and any supplementary information supplied by the applicant available to European Food Safety Authority (EFSA).

Steps taken by EFSA:

- The application was received on 22/12/2008.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- The scientific evaluation procedure started on 22/12/2008.
- During the meeting on 13/03/2009, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to *Lactobacillus plantarum* 299v (DSM 9843) and improve iron absorption.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) 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 plantarum* 299v (DSM 9843) and improve iron absorption.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of *Lactobacillus plantarum* 299v (DSM 9843), a positive assessment of its safety, nor a decision on whether *Lactobacillus plantarum* 299v (DSM 9843) 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 18(4) of Regulation (EC) No 1924/2006.

[2] European Parliament and Council (2006). 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. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

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1. Information provided by the applicant

Applicant's name and address: Probi AB, Ideon Gamma 1, SE-223 70 Lund.

1.1. Food/constituent as stated by the applicant

Lactobacillus plantarum 299v (DSM 9843).

1.2. Health relationship as claimed by the applicant

According to the applicant, "*Lactobacillus plantarum* 299v (DSM 9843) has an ability to attach to human mucosa cells and increase the iron absorption in subjects supplemented with *Lactobacillus plantarum* 299v".

1.3. Wording of the health claim as proposed by the applicant

"*Lactobacillus plantarum* 299v (DSM 9843) improves iron absoption".

1.4. Specific conditions of use as proposed by the applicant

The target population is adults that may be a risk of iron deficiency. According to the applicant, the daily recommended dose is at least 10^9 cfu *Lactobacillus plantarum* 299v a dose that can easily be consumed as part of a balanced diet.

2. Assessment

2.1. Characterisation of the food/constituent

The food/constituent that is the subject of the claim is *Lactobacillus plantarum* 299v (DSM 9843) (hereafter, *L. plantarum* 299v).

The strain L. plantarum 299v (DSM 9843) species identity as well as strain characterisation have been determined by both phenotypic and genotypic methods (Johansson et al., 1993, 1995). Data on the specifications as well as information on stability of the strain in freeze-dried powder or frozen concentrates are provided in the application.

A culture collection number from the German culture collection DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen - DSM 9843) is indicated for the strain.

The Panel considers that the strain that is the subject of the claim (*L. plantarum* 299v) is sufficiently characterised/identified.

2.2. Relevance of the claimed effect to human health

The claimed effect is to improve iron absorption. The target population is adults that are at risk of iron deficiency.

Iron deficiency is one of the most common micronutrient deficiencies with about 30% of world population being anaemic (Ramakrishnan, 2002; WHO, 2008). There is still a significant prevalence of iron deficiency in Europe among pregnant women, children and women in reproductive age (WHO, 1992; Badham *et al*., 2007). The most common consequence of iron deficiency is anaemia.

The Panel considers that improving iron absorption might be beneficial to human health.

2.3. Scientific substantiation of the claimed effect

The applicant performed a literature search using the following databases: PubMed, The Cochrane Library, CINAHL and Web of Science. The following search terms were used: *Lactobacillus plantarum* 299v, *Lactobacillus plantarum*, *Lactobacillus*, *plantarum*, lactic acid bacteria, probiotics, Lp299v and iron absorption. Publications assessing the effect of *L. plantarum* on the absorption of other minerals, phytate degradation and other substances increasing iron absorption were excluded. A total of five articles were identified as relevant by the applicant. Four were human studies focused on the effect of the strain on non-haem iron absorption. Iron absorption was measured in blood by double isotope technique (Hallberg, 1980) and by whole body counting. Two of the studies correspond to published data (Bering et al., 2006 and 2007) and the other two are unpublished studies (Hulthen and Hoppe, 2007). The fifth study provided by the applicant is an unpublished *in vitro* study on iron transport in caco-2 cells (Sandberg, 2006).

Two of the human studies focused on the absorption of iron from an oat gruel product, a food rich in phytates representing a low-Fe bio-availability product (Bering et al., 2006 and 2007). Both studies have a randomised, double-blind, crossover design.

In the study by Bering et al. (2006), 24 women (25±4 years old) with low Fe stores but non-anaemic (serum ferritin concentration 12-40 µg/L and Hb ≥ 110 g/L) were selected. All were non pregnant, non-smokers, non-lactating and not taking vitamin or mineral supplements. Four different products were assessed (test and three control products). The test product (A) consisted in 100g oat gruel fermented with *L. plantarum* 299v (1.1×10^9 cfu/g), control products included: 100 g of (B) pasteurised fermented oat gruel, (C) pH adjusted non-fermented gruel and (D) non-fermented gruel with added organic acids. Two products were administered in each administration period. Each product was consumed for breakfast 2 times for 4 consecutive days (e.g. ABBA), after 18 days wash-out a second period of administration, with the other two products, took place. The authors reported a statistically significant increase on non-haem iron absorption from the test product ($p<0.0001$). Absorption from the pasteurised control was 45% lower and from the non-fermented control was 55% lower than the test product. However, the non-haem iron absorption obtained was very low in all groups.

The Panel notes that no power calculations were reported. In addition, differences in the products composition were observed. Pasteurised gruel contained 19% and 8% lower concentrations of lactic and acetic acids, respectively, than the test product. Non-fermented controls contained 45% and 72% (C) and 60% and 7% (D) lower lactic and acetic content respectively than the test product. The authors indicate that the differences in absorption observed do not appear to be owing to differences on the organic acid composition alone. However, this uncertainty constitutes a limitation of the study, especially as the potential mechanisms involved in the effect of the strain on iron absorption are not known. The non-haem iron absorption obtained was very low in all groups (between 0.5-1.1%). This low concentration corresponds to a total iron amount ranging between 0.01-0.05 mg per product, which may have a very limited relevance from a practical point of view.

In a second study (Bering et al., 2007), 18 women (22±3 years old) with low Fe stores but non-anaemic (serum ferritin concentration 13-29 µg/L and Hb 116-135 g/L) were recruited. All were non pregnant, non-smokers, non-lactating and not taking vitamin or mineral supplements. Sample size was calculated using data from the previous study to obtain a power of 90% to detect a 2% change in iron absorption at a significance level of 0.01. Volunteers consumed, on 2 consecutive days, 100 g of pasteurised fermented oat gruel with added lyophilized viable *L. plantarum* 299v (10^9 cfu/g added 1 day before use) or the same product without *L. plantarum*. After 18 days wash-out the volunteers received the other product. In addition, two capsules with iron were administered together with the gruels to determine colonic absorption of iron. No

significant differences in Fe absorption (1.4 vs. 1.3%) were detected between both products. No significant absorption of Fe was observed in the colon.

Another two human studies, focusing on iron absorption from an iron supplemented fruit drink (high-Fe bio-availability product), have been provided by the applicant (Hulthen and Hoppe 2007, unpublished). Both studies were randomised, single-blinded with crossover design.

In the first study 10 women (22-40 years old) with low Fe stores but non-anaemic were selected. During four consecutive days, volunteers consumed 200 mL fruit drink (supplemented with iron, 2.1 mg/100 mL and ascorbic acid 50mg/100 mL) containing *L. plantarum* 299v (10^9 cfu/200 mL) for two consecutive days and the same fruit drink without *L. plantarum* on the other two days. Iron adsorption from the experimental product was 28.6% vs. 18.5% in the control (p=0.028).

The second study had the same design (11 women 22-40 years old with low Fe stores but non-anaemic) but the test product was supplemented with a higher dose of *L. plantarum* 299v (10^{10} cfu/200mL). In this study the differences observed in iron absorption (29.1 vs. 20.1%) were not statistically significant (p=0.08). When the applicant combined both studies the absorption values for combined experimental products were 28.8% vs. 19.3% for combined control products (p=0.004).

Combining the results of two independent studies, one with significant increase and other with non-significant increase is questionable especially as the studies used two different doses of *L. plantarum* 299v. Also, the positive effects observed in one of the studies could be overestimated when combined with the other study. No dose-effect relationship was observed and the number of volunteers studied was small. In the second study it appears that the test product had higher organic acid contents than the control product.

The Panel considers that the limitations of these studies and conflicting findings restrict their value as a source of data to substantiate the claimed effect.

An unpublished *in vitro* study (Sandberg, 2006) was also provided by the applicant. This study assesses iron transport in caco-2 cells (colonic cells). The five *L. plantarum* strains tested increased iron transport across caco-2 monolayer. This study provides only very limited supportive evidence (Bering et al., 2007).

The Panel concludes that a cause and effect relationship has not been established between the consumption of *L. plantarum* 299v and the improvement of iron absorption.

CONCLUSIONS

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

- "*Lactobacillus plantarum* 299v (DSM 9843)" is sufficiently characterised.
- The claimed effect is to improve iron absorption. The target population is adults that are at risk of iron deficiency. Improving iron absorption might be beneficial to human health.
- A cause and effect relationship has not been established between the consumption of *L. plantarum* 299v and the improvement of iron absorption.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on "*Lactobacillus plantarum* 299v (DSM 9843)" and "improve iron absoption" pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0227-SE). December 2008. Submitted by Probi AB.

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