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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts (ID 945) pursuant to Article 13(1) of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food constituent that is the subject of the health claim is *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845). The Panel considers that *Lactobacillus rhamnosus* GR-1 (ATCC 55826) and *Lactobacillus reuteri* RC-14 (ATCC 55845) are sufficiently characterised.

The claimed effect is “vaginal health/flora”. The target population is assumed to be the general female population. From the clarifications provided by Member States, the Panel assumes that the claimed effect refers to defence against vaginal pathogens by increasing the number of lactobacilli and/or...
Lactobacillus rhamnosus GR-1 (ATCC 55826) in combination with Lactobacillus reuteri RC-14 (ATCC 55845) and defence against vaginal pathogens

decreasing potentially pathogenic bacteria and/or yeasts. The Panel considers that defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts is a beneficial physiological effect.

No references were provided from which conclusions could be drawn for the scientific substantiation of the claim.

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of Lactobacillus rhamnosus GR-1 (ATCC 55826) in combination with Lactobacillus reuteri RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts.

**KEY WORDS**

*Lactobacillus rhamnosus* GR-1 (ATCC 55826), *Lactobacillus reuteri* RC-14 (ATCC 55845), lactobacilli, bacterial vaginosis, yeasts, Nugent score, health claims.
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The consolidated list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 submitted by Member States contains main entry claims with corresponding conditions of use and literature for similar health claims. EFSA has screened all health claims contained in the original consolidated list of Article 13 health claims which was received by EFSA in 2008 using six criteria established by the NDA Panel to identify claims for which EFSA considered sufficient information had been provided for evaluation and those for which more information or clarification was needed before evaluation could be carried out. The clarifications which were received by EFSA through the screening process have been included in the consolidated list. This additional information will serve as clarification to the originally provided information. The information provided in the consolidated list for the health claims, which are the subject of this opinion is tabulated in Appendix C.

ASSESSMENT

1. Characterisation of the food/constituent (ID 945)

The food constituent that is the subject of the health claim is *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845).

Reference to the identification and characterisation of the *Lactobacillus rhamnosus* GR-1 (hereafter *L. rhamnosus* GR-1) strain by different phenotypic and genotypic methods was found in the references provided (Gardiner et al., 2002a; Yeung et al., 2002; Zhong et al., 1998). The Panel considers that *L. rhamnosus* GR-1 is sufficiently characterised. A culture collection number from the American Type Culture Collection (ATCC), ATCC 55826, was provided. The strain was also found in the open access catalogues of the BCCM/LMG (Belgian Co-ordinated Collections of Microorganisms) and NCCB (Netherlands Culture Collection of Bacteria) culture collections under numbers LMG 8153 and NCCB 98073.

The strain *Lactobacillus reuteri* RC-14 (hereafter *L. reuteri* RC-14), initially known as *L. acidophilus* RC-14, reclassified in 1998 as *L. fermentum* RC-14 and finally classified in 2006 by DNA:DNA hybridisation as *L. reuteri* RC-14 (Cadieux et al., 2008), has been characterised at strain level by using different molecular methods (Gardiner et al., 2002b; Zhong et al., 1998). The Panel considers that *L. reuteri* RC-14 is sufficiently characterised. A culture collection number from the ATCC, ATCC 55845, was provided.

The Panel considers that the food constituents, *Lactobacillus rhamnosus* GR-1 (ATCC 55826) and *Lactobacillus reuteri* RC-14 (ATCC 55845), which are the subject of the health claim, are sufficiently characterised.

2. Relevance of the claimed effect to human health (ID 945)

The claimed effect is “vaginal health/flora”. The Panel assumes that the target population is the general female population.

From the clarifications provided by Member States, the Panel assumes that the claimed effect refers to defence against vaginal pathogens by increasing the number of lactobacilli and/or decreasing potentially pathogenic bacteria and/or yeasts.

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Unlike any other anatomical site of the body, most vaginal vaults are dominated by one or more species of *Lactobacillus*. In over 70% of women, vaginal microbiota is dominated by lactobacilli (>50%) (Ling et al., 2010; Ravel et al., 2010; Yamamoto et al., 2009). This microbiota is different from the more complex gut microbiota, where lactobacilli represent less than 3% of the bacterial population (Franks et al., 1998; Lay et al., 2005; Sghir et al., 2000). The diagnosis of bacterial vaginosis (BV) is currently based on the Nugent score (microscopic examination of Gram stained smear or vaginal discharge for bacteria and ‘clue’ cells). Nugent scores are classified into normal (0-3, lactobacilli are present, but not *Gardnerella/Bacteroides* or curved Gram-negative bacilli), intermediate (4-6, colonisation by *Bacteroides*/*Gardenella* and curved Gram-variable rods (*Mobiluncus*)), and BV (7-10, BV with domination of *Gardnerella/Bacteroides* or curved Gram-negative bacilli and absence of lactobacilli).

The Panel considers that defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts is a beneficial physiological effect.

3. **Scientific substantiation of the claimed effect (ID 945)**

The references provided for the scientific substantiation of the claim included narrative reviews and animal studies unrelated to the claimed effect, and *in vitro* studies about, for example, bacterial adhesion properties, cell surface properties, bacterial binding to cell surfaces, and bactericidal activity. Two references could not be evaluated: a conference abstract which did not provide sufficient detail for a full scientific evaluation, and one reference which was not accessible after every reasonable effort had been made to retrieve it. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

Five human intervention studies performed with intra-vaginal capsules were provided. The Panel notes that the intra-vaginal route of administration is not relevant to human nutrition. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

Five human intervention studies on the effects of orally-administered *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14 on Nugent scores and/or numbers of lactobacilli measured in vaginal swabs were provided. Two of these studies were conducted in women receiving antibiotic therapy (Anukam et al., 2006; Reid et al., 2003a), and three were conducted in healthy women (Reid et al., 2001a; 2003b; 2004). The Panel notes that no (Anukam et al., 2006; Reid et al., 2001a; Reid et al., 2003a) or very limited (Reid et al., 2003b; Reid et al., 2004) information about the methods used for statistical analyses was provided in these publications, and therefore did not allow a full scientific evaluation. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

The evidence provided by human studies (Gardiner et al., 2002b; Morelli et al., 2004; Reid et al., 2001b), as well as animal and *in vitro* studies, about the survival of the strains through the gastrointestinal tract and transfer to the vagina is not sufficient to predict the occurrence of an effect of the consumption of *L. rhamnosus* GR-1 (ATCC 55826) in combination with *L. reuteri* RC-14 (ATCC 55845) on defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts in humans.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *L. rhamnosus* GR-1 (ATCC 55826) in combination with *L. reuteri* RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts.
CONCLUSIONS

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

- The food constituents, *Lactobacillus rhamnosus* GR-1 (ATCC 55826) and *Lactobacillus reuteri* RC-14 (ATCC 55845), which are the subject of the health claim, are sufficiently characterised.

- The claimed effect is “vaginal health/flora”. The target population is assumed to be the general female population. Defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts is a beneficial physiological effect.

- A cause and effect relationship has not been established between the consumption of *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts.

DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 (No: EFSA-Q-2008-1732). The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The full list of supporting references as provided to EFSA is available on: http://www.efsa.europa.eu/panels/nda/claims/article13.htm.

REFERENCES


Gardiner GE, Heinemann C, Bruce AW, Beuerman D and Reid G, 2002a. Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA. *Clinical and Diagnostic Laboratory Immunology*, 9, 92-96.


Lactobacillus rhamnosus GR-1 (ATCC 55826) in combination with Lactobacillus reuteri RC-14 (ATCC 55845) and defence against vaginal pathogens


Reid G, Beuerman D, Heinemann C and Bruce AW, 2001a. Probiotic Lactobacillus dose required to restore and maintain a normal vaginal flora. FEMS Immunology and Medical Microbiology, 32, 37-41.


**APPENDICES**

**APPENDIX A**

**BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION**

The Regulation 1924/2006 on nutrition and health claims made on foods\(^6\) (hereinafter "the Regulation") entered into force on 19\(^{th}\) January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

a) the role of a nutrient or other substance in growth, development and the functions of the body; or

b) psychological and behavioural functions; or

c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

(i) based on generally accepted scientific evidence; and

(ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13 (1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

**ISSUES THAT NEED TO BE CONSIDERED**

**IMPORTANCE AND PERTINENCE OF THE FOOD\(^7\)**

Foods are commonly involved in many different functions\(^8\) of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

\(^6\) OJ L12, 18/01/2007

\(^7\) The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.

\(^8\) The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).
It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

**SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE**

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

(a) the claimed effect of the food is beneficial for human health,

(b) a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),

(c) the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,

(d) the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

**WORDING OF HEALTH CLAIMS**

Scientific substantiation of health claims is the main aspect on which EFSA’s opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the requirement to
describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

**TERMS OF REFERENCE**

**HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN’S DEVELOPMENT AND HEALTH**

EFSA should in particular consider, and provide advice on the following aspects:

- Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.
- Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.
- The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

- the claimed effect of the food in the identified function is beneficial.
- a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity
consumed.

➢ where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.

➢ the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

➢ the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

➢ on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.
**APPENDIX B**

**EFSA DISCLAIMER**

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. 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 wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.
APPENDIX C

Table 1. Main entry health claims related to *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845), including conditions of use from similar claims, as proposed in the Consolidated List.

<table>
<thead>
<tr>
<th>ID</th>
<th>Food or Food constituent</th>
<th>Health Relationship</th>
<th>Proposed wording</th>
</tr>
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<tbody>
<tr>
<td>945</td>
<td>Lactobacillus rhamnosus GR-1 (ATCC 55826) and Lactobacillus reuteri RC 14 (ATCC 55845)</td>
<td>Vaginal health/flora&lt;br&gt;Clarification provided&lt;br&gt;Vaginal level of Lactobacilli (Nugent score). Vaginal level of yeast and coliforms:&lt;br&gt;-helps maintain a healthy vaginal microflora by increasing the number of beneficial lactobacilli&lt;br&gt;-helps maintain a healthy vaginal microflora by decreasing yeast and potentially pathogenic bacteria</td>
<td>Helps to restore and maintain a normal vaginal microflora</td>
</tr>
</tbody>
</table>

**Conditions of use**
- Other condition: Tägliche Verzehrmenge: 1*10E9 cfu/Tag
- Other condition: mind.1x10E9 KBE/Tag
- at least 10[9] cfu/day
- at least 10E9 cfu/day; Applicable to both children and adults
**Glossary and Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>BCCM/LMG</td>
<td>Belgian co-ordinated collections of microorganisms/Laboratory of Microbiology Gent</td>
</tr>
<tr>
<td>BV</td>
<td>Bacterial vaginosis</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>NCCB</td>
<td>Netherlands culture collection of bacteria</td>
</tr>
</tbody>
</table>