European Food Safety Authority

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SCIENTIFIC OPINION

LACTORAL and improvement of the general immunity

Scientific substantiation of a health claim related to LACTORAL (a combination of three probiotic strains: *Lactobacillus plantarum, Lactobacillus rhamnosus, Bifidobacterium longum)* and improvement of the general immunity pursuant to Article 14 of Regulation (EC) No 1924/2006[1]

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

(Question No EFSA-Q-2008-477)

Adopted on 28 October 2008 by written procedure

PANEL MEMBERS

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SUMMARY

Following an application from the Institute of Biotechnology, Sera and Vaccines BIOMED S.A. submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Poland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to LACTORAL and improvement of the general immunity.

The scope of the application was proposed to fall under a health claim referring to children's development and health.

The food supplement for which the claim is intended is LACTORAL, a freeze-dried bacterial powder containing *Lactobacillus plantarum* (strain PL02) (34% of the mixture), *Lactobacillus rhamnosus* KL53A (33 %), and *Bifidobacterium longum* PL03 (33%), for oral administration. The total number of bacteria in a dose contained in a sachet (the weight of the sachet was not provided) is claimed by the applicant to be 10^{10} (10 billions) colony forming units (CFUs). The bacterial strains have been identified using phenotypic tests, sequencing of 16S-23S rRNA intergene spacer regions (ITS), and species-specific PCR. The Panel considers that these tests are not sufficient for a proper identification of the bacterial strains, e.g. no data were provided to show that the applied identification methods were able to differentiate between closely

[1] For citation purposes: Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies on a request from the Institute of Biotechnology, Sera and Vaccines BIOMED S.A. on the scientific substantiation of a health claim related LACTORAL and improvement of the general immunity. *The EFSA Journal* (2008) 860, 1-8

related species. Thus the identification of the bacteria remains doubtful. The quality (regarding viability) of the bacterial powder cannot be evaluated as the results of the storage stability studies for LACTORAL have not been provided. The Panel considers that the constituents of the food supplement for which the health claim is made, LACTORAL, have not been sufficiently characterised.

The applicant claims that "LACTORAL is recommended in order to improve the general immunity by maintaining the microbiological balance". The claimed effect of improving the general immunity and the proposed biological mechanism of maintaining the microbiological balance have not been sufficiently defined by the applicant to allow an adequate evaluation of the effect and its impact on health.

The published studies provided to substantiate the health claim relate to a number of different probiotic strains but not to the bacterial strains in LACTORAL. As probiotic effects are strain-specific and dose-dependent these publications cannot be used to substantiate the health claim for LACTORAL. There is only one unpublished *in vitro* study where the effect of the bacterial strains in LACTORAL on leukocytes (including cytokine production) was investigated. *In vitro* studies are not sufficient to predict *in vivo* efficacy in humans. The Panel concludes that a cause and effect relationship has not been established between the consumption of LACTORAL and "improvement of the general immunity by maintaining the microbiological balance".

Based on the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of LACTORAL and the claimed affect.

Key words: Lactoral, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, immunity

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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, Articles 14 to 17 of that 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 Article 15 of that Regulation, an application for authorisation 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 31/03/2008.
- The scope of the application was proposed to fall under a health claim referring to children's development and health.
- During the check for completeness[3] of the application, the applicant was requested to provide missing information on 16/05/2008.
- The applicant provided the missing information on 28/07/2008.
- The scientific evaluation procedure started on 15/08/2008.
- On 28 October 2008 the NDA Panel, after having evaluated the overall data submitted, adopted by written procedure an opinion on the scientific substantiation of a health claim related to LACTORAL and improvement of the general immunity.

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: LACTORAL and improvement of the general immunity.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of LACTORAL, a positive assessment of its safety, nor a decision on whether LACTORAL 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] 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.

[3] In accordance with EFSA "Scientific and Technical guidance for the Preparation and Presentation of the Application for Authorisation of a Health Claim"

ACKNOWLEDGEMENTS

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1. Information provided by the applicant

Applicant's name and address: Institute of Biotechnology, Sera and Vaccines BIOMED S.A.; Al. Sosnowa 8, 30-224 Krakow, Poland

1.1. Food/constituent as stated by the applicant

Food supplement LACTORAL which contains a combination of three probiotic strains: 34% *Lactobacillus plantarum* (PL 02), 33% *Lactobacillus rhamnosus* (KL 53A) and 33% *Bifidobacterium longum* (PL 03).

1.2. Health relationship as claimed by the applicant

The applicant states that "it has been reported that probiotic bacteria exert beneficial effect on health and well-being. Among the possible mechanisms of probiotic supplementation is promotion of a non-immunologic gut defense barrier, which includes the normalisation of increased intestinal permeability and altered gut microecology, or improvement of the intestine's immunologic barrier. Probiotic bacteria from *Lactobacillus* and *Bifidobacterium* genus are part of the intestinal microflora in infants, children and adults. Well balanced intestinal microflora determines proper development of immune system". The applicant claims that "the presence of bifidobacteria and lactobacilli in infant's intestine contribute to the development of immune system and modulate immune activity and epithelial function in the intestine. The probiotic strains enhance natural immunity in human subjects".

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

The applicant proposes the following wording of the health claim: "LACTORAL is recommended in order to improve the general immunity by maintaining the microbiological balance".

1.4 Specific conditions of use as proposed by the applicant

The applicant proposes that "1 to 2 portions a day should be used. Before using, the content of one sachet should be dissolved in little amount of tepid water. Food supplement cannot be used as a substitute for a diversified diet. LACTORAL with strawberry flavour and LACTORAL with nectarine flavour, which additionally contain aroma, should be used in children over 6 months old. Children below 6 months old should be given LACTORAL without flavor, after consulting a doctor".

2. Assessment

2.1. Characterisation of the food/constituent

LACTORAL, a freeze-dried bacterial powder (in milk-saccharose-maltodextrin matrix) is stated to contain *Lactobacillus plantarum* (strain PL02) (34% of the mixture), *Lactobacillus rhamnosus* KL53A (33 %), *Bifidobacterium longum* PL03 (33%), and possibly also aroma (strawberry or nectarine). The total number of bacteria in a dose contained in a sachet (the weight of the sachet was not provided) is claimed to be 10^{10} (10 billions) colony forming units (CFUs). The applicant states that to obtain the claimed effect 1-2 sachets should be consumed per day. The claimed health effect has not been attributed to any specific bacterial strain in the product and no relevant data on the potential mechanism of action *in vivo* for the specific strains have been provided. The bacterial strains in LACTORAL have been identified using the

following tests: phenotypic tests, sequencing of 16S-23S rRNA intergene spacer regions (ITS), and species-specific PCR (Pałuch, unpublished; Heczko and Strus, unpublished). The Panel considers that phenotypic tests alone are not sufficient for a proper identification. The DNA-based identification was not considered sufficient for the following reasons: ITS sequencing is currently not reliable enough because too few *Bifidobacterium* and *Lactobacillus* strains have been sequenced for this region. Thus the reference material available in GenBank for especially *Lactobacillus plantarum* and *Bifidobacterium longum* is too limited for a reliable identification. Furthermore, no analysis of the sequencing results has been provided, only the sequences themselves. Species-specific PCR is reported only in a vague way in a non-published report. Based on the material presented the Panel could not conclude about the specificity of the PCR-method (no controls, i.e. other *Lactobacillus* or *Bifidobacterium* species, were included). Thus the identification of the bacteria, especially regarding *Bifidobacterium longum*, remains doubtful. No data have been provided to show that the identification methods are able to differentiate between closely related species (e.g. within *Lactobacillus plantarum* and *Lactobacillus casei* groups).

The quality (regarding viability) of the bacterial powder cannot be evaluated as the results of the storage stability studies for LACTORAL have not been provided. No specifications were provided on how the viable numbers of the three bacterial strains in the product were determined (culture media and conditions used, how the bacterial strains were differentiated from each other etc.).

The Panel considers that the constituents of the food supplement for which the health claim is made, LACTORAL, have not been sufficiently characterised.

2.2. Relevance of the claimed effect to human health

The applicant claims that "LACTORAL is recommended in order to improve the general immunity by maintaining the microbiological balance". The target population for the food supplement is children. The claimed effect of improving general immunity and the proposed biological mechanism of maintaining the microbiological balance have not been sufficiently defined by the applicant and therefore the effect and its impact on health cannot be evaluated adequately.

2.3. Scientific substantiation of the claimed effect

There is only one unpublished *in vitro* study where the effect of the bacterial strains in LACTORAL on leukocytes (including cytokine production) was studied (Dudek, unpublished). However, *in vitro* studies are not sufficient to predict *in vivo* efficacy in humans (FAO/WHO, 2001).

The Panel concludes that a cause and effect relationship has not been established between the consumption of LACTORAL and the claimed effect.

CONCLUSIONS

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

a) The constituents of LACTORAL are insufficiently characterised regarding the identity, quantity (CFU/g) and stability of the individual bacterial strains.

b) The claimed effect of improving general immunity and the proposed biological mechanism of maintaining the microbiological balance have not been sufficiently

defined by the applicant and therefore its impact on health cannot be evaluated adequately.

c) The Panel concludes that a cause and effect relationship has not been established between the consumption of LACTORAL and "improvement of the general immunity by maintaining the microbiological balance".

DOCUMENTATION PROVIDED TO EFSA

Health claim application on LACTORAL and improvement of the general immunity pursuant to Article 14 of Regulation (EC) No 1924/2006 (EFSA serial No: 0140 b)_PL). July 2008. Submitted by the Institute of Biotechnology, Sera and Vaccines BIOMED S.A.

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GLOSSARY / ABBREVIATIONS

CFUs	Colony forming units
ITS	Intergene spacer regions
PCR	Polymerase chain reaction