SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and maintenance and improvement of intestinal well-being pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

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ABSTRACT

Following an application from Synbiotec S.r.l. submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Italy, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being. The food constituent *Synbio* is sufficiently characterised. The Panel considers that maintenance or improvement of intestinal well-being by increasing intestinal regularity and faecal volume might be a beneficial physiological effect. The target population is healthy adults. The applicant identified 19 references to be pertinent to the health claim. These references included two published articles, one unpublished article, two references on patents, a PhD thesis, six unpublished technical reports on the strains added to different foods and seven communications to conferences. In weighing the evidence, the Panel considers that only one human study evaluated outcomes related to the claimed effect and that the weaknesses of this study (e.g. small sample size, effect of food matrices disregarded, inadequate questionnaire/scale used for measuring the outcomes, and lack of objective outcome measures) limit its value for the scientific substantiation of the claimed effect. The Panel concludes that a cause and effect relationship has not been established between the consumption of *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being by increasing intestinal regularity and faecal volume.

KEY WORDS

Synbio, *Lactobacillus rhamnosus* IMC 501, *Lactobacillus paracasei* IMC 502, maintenance, improvement, intestinal well-being, health claims.

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[1] On request from Synbiotec S.r.l., Question No EFSA-Q-2009-00889, adopted on 10 September 2010.

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SUMMARY

Following an application from Synbiotec S.r.l. submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Italy, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.

The food constituent that is the subject of the health claim is *Synbio*, a combination (1:1) of two bacterial strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®. *Synbio* is available in the form of a lyophilised powder to be added to foods or used as dietary supplement. The identification and characterisation of the strains have been performed by applying phenotypic and genotypic methods. The Panel considers that the food constituent *Synbio* is sufficiently characterised.

The claimed effect is "contributing to maintain and improve intestinal well-being". The target population is healthy adults. The Panel considers that maintenance or improvement of intestinal well-being by increasing intestinal regularity and faecal volume might be a beneficial physiological effect.

The applicant identified 19 references as being pertinent to the health claim. These references included two published articles, one unpublished article, two references on patents, a PhD thesis, six unpublished technical reports on the strains added to different foods and seven communications to conferences. Of these references, only one unpublished study was considered to be pertinent for the scientific substantiation of the claimed effect. The other references were not considered to be pertinent as they focused on strain identification, strain stability in the food products and other technological aspects, on strains survival during gastrointestinal transit or were short communications to conferences that did not provide sufficient detailed information to be used to substantiate the claimed effect.

The unpublished human double-blind controlled clinical trial involved 50 healthy subjects of whom 47 completed the study. Twenty-five subjects were fed six different food products containing the two bacterial strains *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® (1:1) at 10^9 CFU/serving, and 22 subjects were fed placebos, which were the same food products without the strains, for 12 weeks. Microbiological analyses were carried out in stools and bowel habits (intestinal regularity, volume of stools, constipation and flatulence) were self-reported using an arbitrary scale. The faecal levels of *Bifidobacterium* and *Lactobacillus* were reported to be higher in the test group than in the placebo group and the test group reported increases in intestinal regularity and faecal volume after the intervention. The Panel notes that the food products tested were of quite different nature, the number of subjects ingesting each product was not reported and the possible effects of the matrices carrying the strains were not evaluated. The small sample size, the lack of power calculation, and the inadequate questionnaire/scale used for measuring the outcomes were identified as further weaknesses of this study. The Panel notes that the weaknesses of this study and lack of objective outcome measures limit its value for the scientific substantiation of the claimed effect.

In weighing the evidence, the Panel considers that only one human study evaluated outcomes related to the claimed effect and that the weaknesses of this study limit its value for the scientific substantiation of the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being by increasing intestinal regularity and faecal volume.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1924/2006[4] 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 authorisation or 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 the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA:

- The application was received on 10/02/2010.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data.
- The scientific evaluation procedure started on 01/03/2010.
- On 04/06/2010, the NDA Panel agreed on the List of Questions which requests the applicant to supplement additional particulars to accompany the application.
- The applicant submitted the responses to the NDA Panel List of Questions on 22/06/2010.
- During the meeting on 10/09/2010, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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: *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, a positive assessment of its safety, nor a decision on whether *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® is, or is not, classified as

[4] 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.

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.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Synbiotec S.r.l. Via Gentile III da Varano, (62032), Camerino, Italy.

The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

According to the applicant the constituent *Synbio* is a combination (1:1) of two probiotic bacterial strains *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® in the form of lyophilised powder to be added in functional foods.

Health relationship as claimed by the applicant

According to the applicant consuming probiotic functional foods enriched with the *Synbio* constituent, the consumers obtain the effect of a persistence of two probiotic bacterial strains *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® in the intestinal tract favouring the natural regularity and contributing to maintain and improve human intestinal well-being.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wordings for the health claim: "*Synbio* persists in the intestinal tract and favours the natural regularity contributing to maintain and improve human intestinal well-being*"*.

Specific conditions of use as proposed by the applicant

According to the applicant the target population for the intended health claim is adults. The specific study group, in which the evidence was obtained, was composed by healthy human adults (from 23 to 65 years) representative of the target population. A functional food (i.e. yogurt, "ricotta" cheese, "mozzarella" cheese, chocolate, chocolate mousse, ice-cream) containing from 10^7 CFU/g to 10^9 CFU/g of probiotic mixture *Synbio* should be consumed in an amount of 100 g to 10 g to achieve the functional effect. The *Synbio* concentration in food is related to the food common habit consumption (i.e. a common daily intake is about 100 g of "ricotta" cheese, 100 g of ice cream and 10 g of dark chocolate). They can be consumed daily as part of a balanced diet. The category of allergic and intolerant people should avoid using the food containing the constituent for the presence of milk traces in the lyophilised powder (when the cryoprotectant agent is milk). Neither warning for constituent that is likely to present a health risk if consumed to excess, nor other restrictions are present. The final consumer should follow the instructions reported on the label of the probiotic enriched-food packages.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is *Synbio*, a combination (1:1) of two bacterial strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®. *Synbio* is available in the form of a lyophilised powder to be added to foods or used as dietary supplement. The two bacterial strains, *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei*

IMC 502®, were isolated from faeces of healthy elderly people (Silvi et al., 2003). The identification and characterisation of the strains have been done by applying phenotypic and genotypic methods. The genotypic methods applied include sequence analysis of the 16S rRNA gene and RAPD analysis for species and strain identification, respectively (Verdenelli et al., 2009a). The characterisation of the strains has also been published in patents (Cresci et al., 2004, 2005) and in a PhD thesis (Verdenelli, 2007).

The strains were deposited in the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany) culture collection and have the following deposit numbers: DSM 16104 and DSM 16105. The DSMZ has been nominated an International Depositary Authority (IDA) under the Budapest Treaty.

Data on the stability of the strains and their effects on other technological parameters when added to different food products (ice-cream, chocolate mousse, chocolate bar, mozzarella and ricotta cheese, and yogurt) used in intervention human trials were also provided in six unpublished technical reports.

The Panel considers that the food constituent *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "contributing to maintain and improve intestinal well-being". The target population is healthy adults.

The applicant proposes that the claimed effect is the result of improvements on bowel physiological functions, including intestinal regularity and faecal volume.

The Panel considers that maintenance or improvement of intestinal well-being by increasing intestinal regularity and faecal volume might be a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

According to the applicant a literature search in PubMed, using search terms such as "IMC 501 and IMC 502", was performed.

The applicant identified 19 references as being pertinent to the health claim. These references included two published articles (Silvi et al., 2003; Verdenelli et al., 2009a), one unpublished article by Verdenelli et al., two references on patents (Cresci et al., 2004, 2005), a PhD thesis (Verdenelli, 2007), six unpublished technical reports on the strains added to different foods (yogurt, "ricotta" cheese, "mozzarella" cheese, chocolate, chocolate mousse and ice-cream) and seven communications to conferences (Cecchini et al., 2009; Ghelfi et al., 2005; Silvi et al., 2009; Verdenelli et al., 2008a, 2008b, 2009b, 2009c). Of these references, only the unpublished study by Verdenelli et al. was considered as being pertinent for the scientific substantiation of the claimed effect. The other references were not considered to be pertinent as they focused on strain identification (Silvi et al., 2003), on strain stability in the food products and other technological aspects (unpublished technical reports), on strains survival during gastrointestinal transit (Verdenelli et al., 2009a) or were short communications to conferences that did not provide sufficient detailed information to be used to substantiate the claimed effect (Cecchini et al., 2009; Ghelfi et al., 2005; Silvi et al., 2009; Verdenelli et al., 2008a, 2008b, 2009b, 2009c). The patent documents and the PhD thesis included the same data as those provided by the published and unpublished studies.

The unpublished study by Verdenelli et al. was a human double-blind controlled clinical trial, involving 50 healthy subjects (from 23 to 65 years old) of whom 47 completed the study. Twenty-five

subjects were fed six different food products (yoghurt, "ricotta" cheese," mozzarella" cheese, chocolate, chocolate mousse and ice-cream) containing the two bacterial strains *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® (1:1) at 10^9 CFU/serving, and 22 subjects were fed placebos, which were the same food products without the strains, for 12 weeks. Microbiological analyses were carried out in stools and bowel habits (intestinal regularity, volume of stools, constipation and flatulence) were self-reported using an arbitrary scale. The faecal levels of *Bifidobacterium* and *Lactobacillus* were reported to be higher in the test group than in the placebo group and the test group reported increases in intestinal regularity and faecal volume after the intervention. The Panel notes that the food products tested were of quite different nature, the number of subjects ingesting each product was not reported and the possible effects of the matrices carrying the strains were not evaluated. Sample size was small and no power calculation was reported. No reference to validation of the scale used was given in the original article. After a request for clarification, the applicant indicated that intestinal regularity and faecal volume measurements were based on an adapted version of the validated Birmingham IBS symptom questionnaire (Roalfe et al., 2008). The adaptation was not explained, there was no indication of which items of the IBS symptom questionnaire were used for setting the scale employed and no item of the quoted questionnaire was related to faecal volume measurements. The validation of the questionnaire for this study, as described by the applicant, consisted of ensuring the comprehensiveness of the questions/scale, but correlations with existing reference methods and/or objective measures (e.g. weight of stools, number of depositions, etc.) were not provided. The Panel notes that the weaknesses of this study, including small sample size and the lack of power calculation, possible effect of food matrices disregarded, inadequate questionnaire/scale used for measuring the outcomes and lack of objective outcome measures, limit its value for the scientific substantiation of the claimed effect.

In weighing the evidence, the Panel considers that only one human study evaluated outcomes related to the claimed effect and that the weaknesses of this study (e.g. small sample size, lack of power calculation, possible effect of food matrices disregarded, inadequate questionnaire/scale used for measuring the outcomes and lack of objective outcome measures) limit its value for the scientific substantiation of the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being by increasing intestinal regularity and faecal volume.

CONCLUSIONS

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

- The food constituent *Synbio*, a combination (1:1) of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is "contributing to maintain and improve intestinal well-being". The target population is healthy adults. Maintenance or improvement of intestinal well-being by increasing intestinal regularity and faecal volume might be a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being by increasing intestinal regularity and faecal volume.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on *Synbio*, a combination of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502®, and contribution to maintaining and improving intestinal well-being pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0263_IT). February 2010. Submitted by Synbiotec S.r.l.

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GLOSSARY/ABBREVIATIONS

CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
IBS	Irritable Bowel Syndrome
IDA	International Depositary Authority
RAPD	Random Amplified Polymorphic DNA