SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 16 June 2011, replaces the earlier version published on 18 October 2010[4].

ABSTRACT

Following an application from Yakult Europe B.V., submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the Netherlands, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim based on newly developed scientific evidence and including a request for the protection of proprietary data related to *Lactobacillus casei* strain Shirota (*Lc*S) and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences. The food constituent, *Lc*S, which is the subject of the health claim, is sufficiently characterised. The Panel considers that maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences is a beneficial physiological effect. The applicant identified a total of 12 references as being pertinent to the health claim. These included nine human intervention trials and three animal studies. In weighing the evidence, the Panel took into account that there was no human study from which conclusions could be drawn for an effect of *Lc*S consumption on upper respiratory tract infections, that one human study did not support an effect of *Lc*S consumption on the immune response to influenza vaccination, and that there was a lack of evidence for an effect of *Lc*S consumption on the immune system that could relate to the defence of the upper respiratory tract against pathogens. The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lc*S and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences. © European Food Safety Authority, 2010

[1] On request of the Competent Authority of The Netherlands following an application from Yakult Europe B.V., Question No EFSA-Q-2010-00137, adopted by written procedure on 07 October 2010.

[2] Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. One member of the Panel did not participate in the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen for the preparatory work on this scientific opinion.

[4] After publication of this opinion, the applicant notified to EFSA that there was a misinterpretation of the statistical analysis of the data of the Gleeson et al. study. The opinion has been changed accordingly. This change does not affect the conclusions of the opinion. Where changes have been made to the opinion footnotes have been included to indicate the original text.

For citation purposes: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(10):1860. [15 pp.]. doi:10.2903/j.efsa.2010.1860. Available online: www.efsa.europa.eu/efsajournal.htm

KEY WORDS

Lactobacillus casei, fermented milk product, upper respiratory tract infections, health claims.

SUMMARY

Following an application from Yakult Europe B.V., submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the Netherlands, 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 casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences.

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 *Lactobacillus casei* strain Shirota (*Lc*S). The identification and characterisation of the strain has been performed by applying phenotypic and genotypic methods. The Panel considers that the food constituent, *Lactobacillus casei* strain Shirota, which is the subject of the health claim, is sufficiently characterised.

The claimed effect is "maintenance of immune defences, including upper respiratory tract defence". The target population is the general population. The Panel considers that maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences is a beneficial physiological effect.

A total of 12 references, which included nine human intervention studies and three animal studies, were considered to be pertinent by the applicant for either the scientific substantiation of the claim (i.e. maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences) or as supporting evidence for an effect of *Lc*S on the immune system that could relate to the defence of the upper respiratory tract against pathogens.

In a randomised, double-blind, placebo-controlled intervention, 84 endurance athletes were allocated to consume daily either two bottles of fermented milk containing at least 6.5 x 10^9 CFU of live *Lc*S per bottle or a similar placebo drink without bacteria for 16 weeks during the winter training period. The primary outcome of the study was incidence of upper respiratory tract infections (URTI). Secondary outcomes included severity and duration of URTI episodes, symptoms of gastrointestinal discomfort, and changes in concentrations of several immune markers in biological samples. Subjects were asked to complete weekly a self-administered health questionnaire by rating a series of symptoms as light, moderate or severe. The Panel notes that the questionnaire used was constructed from previously validated questionnaires to assess the incidence of URTI but no information on validation of the specific questionnaire used in the present study was provided. The Panel considers that it has not been shown that this approach provides an appropriate measure of the presence of URTI. A total of 26 subjects (31 %) dropped out during the study. Data analysis was performed in the population of completers only. The Panel considers that the high dropout rate was not taken into account appropriately in the analysis of the data[5]. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim

A randomised, double-blind, placebo-controlled pilot trial was designed to test the feasibility of a daily intervention with two bottles of fermented milk containing at least 6.5 x 10^9 CFU of live *Lc*S per bottle in an elderly nursing home population. The study report indicates that the study size (n=38 per group) was not adequate to detect significant differences between *Lc*S and placebo groups for incidence of URTI. The Panel agrees that this feasibility study was underpowered to assess the effects of *Lc*S consumption on the incidence of URTI, and that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Subsequent to this pilot study, a multicentre, randomised, double-blind, placebo-controlled trial was conducted in 737 healthy elderly nursing home residents. Subjects were randomly assigned to consume daily two bottles of fermented milk containing at least 6.5 x 10^9 CFU of live *Lc*S per bottle

[5] 'for the entire duration of the study' has been deleted

or a similar placebo drink without bacteria for 176 days. On day 21, all subjects received an influenza vaccine. Primary outcomes of the study were cumulative incidence of upper respiratory tract influenza-like infections and specific IgG antibody levels in serum. Subjects and nursing home staff were asked to record symptoms of running nose, sore throat, fever and cough each day. URTI was defined as the presence of one or more of the symptoms above. The Panel notes that the symptoms used to define the occurrence of URTI (i.e. running nose, sore throat, fever and cough) are non-specific, and that the presence of one or more of these symptoms is not an appropriate measure of the occurrence of URTI in the study population. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claimed effect.

In the same study, the effect of consumption of the *Lc*S drink on the response to influenza vaccination, protective antibody titres, seroconversion and seroprotection rates in the intervention group was compared to the placebo group at days 50 and 176. No significant differences in IgG titres, seroconversion and seroprotection rates between groups were observed. Data were also analysed according to the participants' ages, taking 80 years as a cut off. The Panel notes that this comparison was not pre-planned in the study protocol and that no scientific justification was provided for it. The Panel considers that this study does not support an effect of *Lc*S consumption on protective IgG titres, seroconversion and seroprotection rates after influenza vaccination in humans.

The remaining publications provided by the applicant reported on effects on the immune system that could relate to the defence of the URT against pathogens. These studies include six human intervention studies and three studies in animal models.

Three double-blind placebo-controlled intervention studies in healthy human subjects investigated the effect of *Lc*S consumption on natural killer (NK) cell activity, among other immune parameters. In one study, no direct comparison between the intervention and placebo group on NK cell activity was provided. The Panel considers that no conclusions can be drawn from this study with regard to effects on the immune system that could relate to the defence of the URT against pathogens. In a second trial a significant increase in NK cell activity and a significantly increased interleukin (IL)-12 synthesis by peripheral blood mononuclear cells were observed *ex vivo* following the administration of *Lc*S. However, in a third study *Lc*S-fermented milk did not significantly influence several immune markers. The Panel notes that the results from these two studies do not show a consistent effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

One double-blind placebo-controlled study was conducted in subjects with seasonal allergic rhinitis to assess the effects of *Lc*S consumption on antigen-induced IL-5, IL-6 and IFN-γ, specific IgE and specific IgG production. The Panel notes that the evidence provided does not establish that changes in these immune parameters in subjects with seasonal allergic rhinitis are related to the defence of the URT against pathogens in the general population.

The remaining human studies were one-arm, uncontrolled interventions in subjects with human T-cell lymphotropic virus type 1 (HTLV-1) infection and alcoholic liver cirrhosis, respectively. The Panel considers that no conclusions can be drawn from these studies to support an effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

Three studies examined the effects of *Lc*S administration on immune function in animal models. The Panel considers that no conclusions can be drawn from these studies to support an effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

In weighing the evidence the Panel took into account that there was no human study from which conclusions could be drawn for an effect of *Lc*S consumption on URTI, that one human study did not support an effect of *Lc*S consumption on the immune response to influenza vaccination, and that there was a lack of evidence for an effect of *Lc*S on the immune system that could relate to the defence of the URT against pathogens.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences.

TABLE OF CONTENTS

Abstract 1
Summary 3
Table of contents 6
Background as provided by the European Commission 7
Terms of reference as provided by the European Commission 7
EFSA Disclaimer 7
Information provided by the applicant 8
Assessment 8
1. Characterisation of the food/constituent 8
2. Relevance of the claimed effect to human health 9
3. Scientific substantiation of the claimed effect 9
Conclusions 12
Documentation provided to EFSA 12
References 12
Glossary and Abbreviations 15

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1924/2006[6] 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, Article 13(5) of this Regulation lays down provisions for the 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 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).

Steps taken by EFSA:

- The application was received on 26/03/2010.
- 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 30/03/2010.
- On 21/05/2010, the NDA Panel agreed on a list of questions which requested the applicant to provide additional particulars to accompany the application by 26/06/2010.
- The applicant submitted the responses to the NDA Panel's list of questions on 25/06/2010.
- During the meeting of 08-10/09/2010, the NDA Panel discussed the draft opinion and agreed to adopt the opinion by written procedure.
- On 07/10/2010, 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 *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences.

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: *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences.

EFSA DISCLAIMER

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

[6] 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.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Yakult Europe B.V., Head Office and Production, Schutsluisweg 1, 1332 EN, Almere, The Netherlands.

Food/constituent as stated by the applicant

According to the applicant the food constituent which is the subject of the health claim is live *Lactobacillus casei* strain Shirota.

Health relationship as claimed by the applicant

According to the applicant the health relationship refers to the maintenance of immune defences, including upper respiratory tract (URT) defence, measured as a change in the incidence and duration of URT infections in combination with a corresponding change in immune parameters.

Wording of the health claim as proposed by the applicant

The following wording is proposed by the applicant: "Daily consumption of live *Lactobacillus casei* strain Shirota as present in a fermented milk product helps maintain the upper respiratory tract defences by helping to support immune functions." Alternative wordings with the same meaning have been proposed by the applicant.

Specific conditions of use as proposed by the applicant

According to the applicant at least 1.3 x 10^{10} live *Lactobacillus casei* strain Shirota in 130 mL of a fermented milk product should be consumed to obtain the claimed effect. The given amount of *Lactobacillus casei* strain Shirota is commercially available in two 65 mL bottles of Yakult®. The product is intended to be used on a daily basis in addition to the habitual diet. The target population is the general population.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent, which is the subject of the health claim, is live *Lactobacillus casei* strain Shirota (*Lc*S).

L. casei YIT 9029 (or *L. casei* strain Shirota) has been deposited in the Culture Collection of the Agency of Industrial Science and Technology, Japan (formerly Fermentation Research Institute) under deposit number FERM BP-1366 in 1981. The Judicial Commission of the International Committee on Systematics of Bacteria has recently reclassified *Lc*S as belonging to the species *L. paracasei* whereas *L. zeae* strains (ATCC 393 and ATCC 15820) were classified as *L. casei* (JCICSB, 2008). Accordingly, *Lc*S, although commonly referred to as a *L. casei* strain, should now be referred to as a *L. paracasei* strain.

The bacterium *Lc*S has been characterised by phenotypic and genotypic methods (Watanabe and Mike, 1999). The strain is a Gram-positive, catalase-negative, non-motile, non-spore-forming and facultatively anaerobic bacterium. The genotypic characterisation of the strain *Lc*S at species level included 16S rRNA gene sequencing and DNA-DNA hybridisation. The entire genome of *Lc*S has been determined (Sato et al., 2004) but its actual base sequence has been kept confidential and only some DNA sequences have been deposited in gene databases under the accession numbers X02734 (Shimizu-Kadota et al., 1985), AB023773 (Shimizu-Kadota et al., 2000), and AB470649 (Yasuda et al., 2008). In addition, the strain has been identified and characterised at strain level by different molecular methods including random-amplified polymorphic DNA (Fujimoto et al., 2008), direct genome DNA sequence comparison (Sato et al., 2004), and pulse-field gel electrophoresis (Tuohy et

al., 2007). The strain has also been reported to be detected using specific monoclonal antibodies (Yuki et al., 1999).

A fermented milk product containing the food constituent live *Lc*S is commercially available under the brand name Yakult® (Jpn Pat. No 183156, 1945). Each bottle contains 65 mL with a minimum number of viable *Lc*S of 1 x 10^8 CFU/mL. The ingredients of Yakult (48 kcal per bottle) are water, skimmed milk, glucose-fructose syrup, sucrose, aroma (citrus) and live *Lc*S. An energy-reduced version of Yakult (Yakult Light, 27 kcal per bottle), with the same specifications with respect to the number of viable cells of *Lc*S, has been commercialised in Europe. The quality control system used by Yakult is ISO 9001 and HACCP (certified). Yakult/Yakult Light should be kept in the refrigerator below 7°C. The shelf life of Yakult/Yakult Light is 31 days. The applicant indicates that the minimum number of live cells of *Lc*S guaranteed at the end of shelf-life is 1 x 10^8 CFU/mL, equivalent to 6.5 x 10^9 CFU per 65 mL bottle (Kawami et al., 1999). Other studies reported numbers of 9.2 x 10^8 CFU/mL in the Yakult product (Reuter, 1997) and 1.4 x 10^8 CFU/mL (Consumentenbond, 2006) at the end of shelf-life, which was very close to the value obtained immediately after manufacturing (1.5 x 10^8 CFU/mL).

The Panel considers that the food, *Lactobacillus casei* strain Shirota, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "maintenance of immune defences, including upper respiratory tract defence". The target population is the general population.

Changes in the upper respiratory tract (URT) defence against pathogens can be measured as changes in the incidence and duration of URT infections. Supporting evidence for the participation of immune responses in the defence mechanism can be obtained from corresponding changes in appropriate immune markers.

The Panel considers that maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant provided information on the literature search strategy. Pubmed was used as the only literature database. The keywords used to identify publications regarding *Lc*S and maintainance of the upper respiratory tract defence against pathogens are given.

A total of nine references reporting on human intervention studies (Gleeson et al., 2009, unpublished; van Puyenbroeck et al., 2009, unpublished; van Gils et al., 2009, unpublished; Spanhaak et al., 1998; Nagao et al., 2000; Takeda et al., 2006; Ivory et al., 2008; Matsuzaki et al., 2005; Stadlbauer et al., 2008) and three studies in animal models (Hori et al., 2001, 2002; Yasui et al., 2004) were considered by the applicant to be pertinent for either the scientific substantiation of the claimed effect (i.e. maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences) or as supporting evidence for the participation of the immune system in the defence mechanism of the URT against pathogens.

Three controlled interventions in humans reported on the effects of *Lc*S in the incidence of URT infections (URTI) in athletes (Gleeson et al., 2009, unpublished) and elderly subjects (van Puyenbroeck et al., 2009, unpublished; van Gils et al., 2009, unpublished).

In a randomised, double-blind, placebo-controlled intervention, 84 endurance athletes (30 females, age range 18-55 years) were allocated to consume daily either two bottles of fermented milk containing at least 6.5 x 10^9 CFU of live *Lc*S per bottle (n=42) or a similar placebo drink without bacteria (n=42) for 16 weeks during the winter training period (Gleeson et al., 2009, unpublished). The primary outcome of the study was the incidence of URTI. Power calculations were performed and it was estimated that

32 subjects per group were required to observe a difference between the intervention and control groups of 2.0±1.0 URTI episodes during the winter months (i.e. a 30 % difference). Secondary outcomes included severity and duration of URTI episodes, symptoms of gastrointestinal discomfort, and changes in concentrations of several immune markers in biological samples (e.g. saliva IgA, blood cell count, blood immunoglobulins, blood lymphocyte subsets and antigen-stimulated whole blood culture production of cytokines). Subjects were asked to complete weekly a self-administered health questionnaire by rating a series of symptoms as light, moderate or severe (i.e. sore throat, catarrh in the throat, runny nose, cough, repetitive sneezing, fever, persistent muscle soreness, joint aches and pain, weakness, headache and loss of sleep), which were coded as 1 (light), 2 (moderate) or 3 (severe) by the investigators. The total symptom score for every subject for each week was calculated by multiplying the total number of days each symptom was experienced by the numerical ratings attributed to that symptom. A total symptom score of ≥12 was taken to indicate the presence of URTI. The Panel notes that the questionnaire used was constructed from previously validated questionnaires to assess the incidence of URTI but no information on the validation of the specific questionnaire used in the present study was provided. The Panel considers that it has not been shown that this approach provides an appropriate measure of the presence of URTI. A total of 26 subjects (31%) dropped out during the study (10 (24 %) in the intervention group *versus* 16 (38%) in the placebo group). Reasons for withdrawal included frequent travel and impossibility to carry the liquid supplements, injury and persistent illness. However, the number of dropouts for each reason was not reported. Data analysis was performed in the population of completers only. Upon a request from the Panel for clarification on how dropouts were taken into account in the analysis of data, the applicant provided an analysis of the data in relation to the incidence of URTI, including 65 subjects who completed at least 8 weeks of the study out of 84 subjects randomised[7]. The Panel considers that the high dropout rate was not taken into account appropriately in the analysis of the data (for primary and secondary outcomes). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim owing to the aforementioned methodological limitations of this study.

A randomised, double-blind, placebo-controlled pilot trial was designed to test the feasibility of a daily intervention with two bottles of fermented milk containing at least 6.5 x 10^9 CFU of live *Lc*S per bottle in an elderly nursing home population (van Gils et al., 2009, unpublished). The study report indicates that the study size (n=38 per group) was not adequate to detect significant differences between *Lc*S and placebo groups for incidence of URTI. The Panel agrees that this feasibility study was underpowered to assess the effects of *Lc*S consumption on the incidence of URTI and that no conclusions can be drawn from this study for the scientific substantiation of the claim.

Subsequent to this pilot study, a multicentre, randomised, double-blind, placebo-controlled trial was conducted in 737 healthy elderly nursing home residents aged 65 years and older (van Puyenbroeck et al., 2009, unpublished). Subjects were randomly assigned to consume daily two bottles of fermented milk containing at least 6.5 x 10^9 CFU of live *Lc*S per bottle (n=375) or a similar placebo drink without bacteria (n=362) for 176 days. On day 21, all subjects received an influenza vaccine. Primary outcomes of the study were cumulative incidence of upper respiratory tract influenza-like infections (URTI) (outcome measure used to perform power calculations) and specific IgG antibody levels in serum. Subjects and nursing home staff were asked to record symptoms of running nose, sore throat, fever and cough each day. Questionnaires were evaluated by investigators every three weeks. URTI was defined as the presence of one or more of the symptoms above. A total of 183 participants (25 %) dropped out during the study (90 in the control group and 93 in the intervention group). Reasons for withdrawal were clearly stated in the study protocol. As many of the participants' diaries were not complete, it was assumed that data were missing at random and the probability of URTI symptoms each day was used as the outcome variable (instead of the initially planned cumulative incidence of URTI). The Panel notes that the symptoms used to define the occurrence of URTI (i.e. running nose, sore throat, fever and cough) are non-specific and that the presence of one or more of these symptoms

[7] Original text: Upon a request from the Panel for clarification on how dropouts were taken into account in the analysis of data, the applicant provided an intention-to-treat analysis of the data in relation to the incidence of URTI only during the first eight weeks of the study (intermediate results) but not for the entire duration of the study (16 weeks).

is not an appropriate measure of the occurrence of URTI in the study population. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

In the same study, the effect of consumption of the *Lc*S drink on the response to influenza vaccination, protective antibody titres, seroconversion and seroprotection rates in the intervention group was compared to the placebo group at days 50 and 176. One hundred and seventy two participants were considered to be seroprotected at baseline (anti-influenza antibody titres >40) and were therefore excluded from serological analyses. No significant differences in specific IgG titres, seroconversion and seroprotection rates between groups were observed. Data were also analysed according to the participants' ages, taking 80 years as a cut-off. The Panel notes that this comparison was not pre-planned in the study protocol and that no scientific justification has been provided for it. The Panel considers that this study does not support an effect of *Lc*S consumption on mean protective IgG titres, seroconversion and seroprotection rates after influenza vaccination in humans.

The remaining publications provided by the applicant reported on effects of the immune system that could relate to the defence of the URT against pathogens. These studies include six human intervention studies (Spanhaak et al., 1998; Nagao et al., 2000; Takeda et al., 2006; Ivory et al., 2008; Matsuzaki et al., 2005; Stadlbauer et al., 2008) and three studies in animal models (Hori et al., 2001, 2002; Yasui et al., 2004).

Three double-blind, placebo-controlled intervention studies in healthy human subjects investigated the effect of *Lc*S consumption on natural killer (NK) cell activity, among other immune parameters (Nagao et al., 2000; Takeda et al., 2006; Spanhaak et al., 1998). In the study by Nagao et al. (2000), no direct comparison between the intervention (4×10^{10} CFU of *Lc*S per bottle given once daily, n=9) and placebo (no *Lc*S, n=8) group on natural killer (NK) cell activity was provided. The Panel considers that no conclusions can be drawn from this study with regard to effects on the immune system that could relate to the defence of the URT against pathogens. In the placebo-controlled cross-over trial by Takeda et al. (2006), subjects (n=10) consumed in a cross-over design fermented milk containing 4×10^{10} CFU of *Lc*S per bottle given once daily, or fermented milk not containing the bacteria, for three weeks each. A significant increase in NK cell activity and a significantly increased interleukin (IL)-12 synthesis by peripheral blood mononuclear cells (PBMC) were observed *ex vivo* following the administration of *Lc*S. However, in the placebo-controlled, parallel study by Spanhaak et al. (1998), the consumption three times daily of one bottle of fermented milk containing *Lc*S at a dose of 10^{11} CFU per bottle did not significantly influence several immune markers (including NK cell activity, phagocytic activity and cytokine production) in healthy males (n=10) as compared to the control drink (n=10). The Panel notes that the results from these two studies do not show a consistent effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

One double-blinded placebo-controlled study was conducted in subjects with seasonal allergic rhinitis to assess the effects of *Lc*S consumption on antigen-induced IL-5, IL-6, IFN-γ, specific IgE and specific IgG production (Ivory et al., 2008). The Panel notes that the evidence provided does not establish that changes in these immune parameters in subjects with seasonal allergic rhinitis are related to the defence of the URT against pathogens in the general population.

The remaining human studies were one-arm, uncontrolled interventions in subjects with human T-cell lymphotropic virus type 1 (HTLV-1) infection (Matsuzaki et al., 2005) and alcoholic liver cirrhosis (Stadlbauer et al., 2008), respectively. The Panel considers that no conclusions can be drawn from these studies to support an effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

Three studies examined the effects of *Lc*S administration on immune function in animal models. In one of the studies, *Lc*S was administered directly in the nose (Hori et al., 2001), a route of exposure which is not relevant for oral consumption. Other animal studies involved oral administration of *Lc*S to mice, and investigated titres of influenza virus after infection in addition to several parameters

related to the immune system such as NK activity and IL-12, IFN-γ and TNF-α production (Hori et al., 2002; Yasui et al., 2004). The Panel considers that no conclusions can be drawn from these studies to support an effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

In weighing the evidence, the Panel took into account that there was no human study from which conclusions could be drawn for an effect of *Lc*S consumption on URTI, that one human study did not support an effect of *Lc*S consumption on the immune response to influenza vaccination, and that there was a lack of evidence for an effect of *Lc*S consumption on the immune system that could relate to the defence of the URT against pathogens.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences.

CONCLUSIONS

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

- The food, *Lactobacillus casei* strain Shirota, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is "maintenance of immune defences, including upper respiratory tract defence". The target population is the general population. Maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of *Lactobacillus casei* strain Shirota and maintenance of the upper respiratory tract defence against pathogens by maintaining immune defences.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on *Lactobacillus casei* strain Shirota and maintenance of immune defences, including upper respiratory tract defence pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0273_NL). March 2010. Submitted by Yakult Europe B.V.

REFERENCES

Consumentenbond, 2006. Probiotische zuiveldranken.

Fujimoto J, Matsuki T, Sasamoto M, Tomii Y and Watanabe K, 2008. Identification and quantification of *Lactobacillus casei* strain Shirota in human feces with strainspecific primers derived from randomly amplified polymorphic DNA. International Journal of Food Microbiology, 126, 210-215.

Gleeson M, Bishop NC, Oliveira M and Tauler P, 2009. Daily probiotic (Lactobacillus casei Shirota) reduces infection incidence in athletes (unpublished).

Hori T, Kiyoshima J, Shida K and Yasui H, 2002. Augmentation of cellular immunity and reduction of influenza virus titer in aged mice fed Lactobacillus casei strain Shirota. Clinical and Diagnostic Laboratory Immunology, 9, 105-108.

Hori T, Kiyoshima J, Shida, K and Yasui H, 2001. Effect of intranasal administration of Lactobacillus casei Shirota on influenza virus infection of upper respiratory tract in mice. Clinical and Diagnostic Laboratory Immunology, 8, 593-597.

Ivory K, Chambers SJ, Pin C, Prieto E. Arques JL and Nicoletti C, 2008. Oral delivery of Lactobacillus casei Shirota modifies allergen-induced immune responses in allergic rhinitis. Clin. Exp. Allergy, 38, 1282-1289.

JCICSB (Judicial Commission of the International Committee on Systematics of Bacteria), 2008. The type strain of *Lactobacillus casei* is ATCC 393, ATCC 334 cannot serve as the type because it represents a different taxon, the name *Lactobacillus paracasei* and its subspecies name are not rejected and the revival of the name '*Lactobacillus zeae*'contravenes Rules 51b (1) and (2) of the International Code of Nomenclature of Bacteria. Opinion 82. International Journal of Systematic and Evolutionary Microbiology, 58, 1764-1765.

Kawami K, 1999. Part I Properties of Lactobacillus casei, Section 6 Culture and fermentation. In: *Lactobacillus casei* Shirota – Intestinal flora and human health. Ed Yakult Central Institute for Microbiological Research, Kyoto, 87-92.

Matsuzaki T, Saito M, Usuku K, Nose H, Izumo S, Arimura K and Osame M, 2005. A prospective uncontrolled trial of fermented milk drink containing viable Lactobacillus casei strain Shirota in the treatment of HTLV-1 associated myelopathy/tropical spastic paraparesis. Journal of the Neurological Sciences, 237, 75-81.

Nagao F, Nakayama M, Muto T and Okumura K, 2000. Effects of a fermented milk drink containing Lactobacillus casei strain Shirota on the immune system in healthy human subjects. Bioscience, Biotechnology, and Biochemistry, 64, 2706-2708.

Reuter G, 1997. Present and future of probiotics in Germany and in Central Europe. Bioscience and Microflora, 16, 43-51.

Sato T, Shirasawa Y, Kiwaki M and Sato S, 2004. Genome analysis of *Lactobacillus casei* strain Shirota and *Bifidobacterium breve* strain Yakult [in Japanese]. Seibutsu-kogakukaishi, 82, 421-422.

Shimizu-Kadota M, Kiwaki M, Hirokawa H and Tsuchida N, 1985. ISL1: a new transposable element in *Lactobacillus casei*. Molecular and General Genetics, 200, 193-198.

Shimizu-Kadota M, Kiwaki M, Sawaki S, Shirasawa Y, Shibahara-Sone H and Sako T, 2000. Insertion of bacteriophage phiFSW into the chromosome of *Lactobacillus casei* strain Shirota (S-1): characterization of the attachment sites and the integrase gene. Gene, 249, 127-134.

Spanhaak S, Havenaar R and Schaafsma G, 1998. The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans. European Journal of Clinical Nutrition, 52, 899-907.

Stadlbauer V, Mookerjee, RP, Hodges SJ, Wright G, Davies NA and Jalan R, 2008. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. Journal of Hepatology, 48, 945-951.

Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M and Okumura K, 2006. Interleukin-12 is involved in the enhancement of human natural killer cell activity by Lactobacillus casei Shirota. Clinical and Experimental Immunology, 146, 109-115.

Tuohy KM, Pinart-Gilberga M, Jones M, Hoyles L, McCartney AL and Gibson GR, 2007. Survivability of a probiotic *Lactobacillus casei* in the gastrointestinal tract fo healthy human volunteers and its impact on the faecal microflora. Journal of Applied Microbiology, 102, 1026-1032.

van Gils L, Dirven K, Coenen S and van Rouen P, 2009. Pilot study to investigate the feasibility of a study protocol investigating the adjuvant effect of *Lactobacillus casei* Shirota on the efficacy of influenza vaccination in elderly (unpublished).

van Puyenbroeck K, Beunckens C, Coenen S, Michiels B, Hens N, Molenberghs G, van Royen P and Verhoeven V, 2009. Efficacy of daily intake of L.casei Shirota on respiratory symptoms and influenza vaccination immune response: a randomized double-blind placebo-controled trial in healthy elderly nursing home residents (unpublished).

Watanabe T and Mike A, 1999. Part I Properties of Lactobacillus casei, Section 2 Taxonomy. In: *Lactobacillus casei* Shirota – Intestinal flora and human health. Ed Yakult Central Institute for Microbiological Research, Kyoto, 3-23.

Yasuda E, Serata M and Sako T, 2008. Suppressive effect on activation of macrophages by *Lactobacillus casei* strain Shirota genes determining the synthesis of cell wall associated polysaccharides. Applied and Environmental Microbiology, 74, 4746-4755.

Yasui H, Kiyoshima J and Hori T, 2004. Reduction of influenza virus titer and protection against influenza virus infection in infant mice fed Lactobacillus casei Shirota. Clinical and Diagnostic Laboratory Immunology, 11, 675-679.

Yuki N, Watanabe K, Mike A, Tagami Y, Tanaka R, Ohwaki M and Morotomi M, 1999. Survival of a probiotic, *Lactobacillus casei* strain Shirota, in the gastrointestinal tract:selective isolation from faeces and identification using monoclonal antibodies. International Journal of Food Microbiology, 48, 51-57.

GLOSSARY AND ABBREVIATIONS

ATCC	American Type Culture Collection
CFU	Colony Forming Unit
DNA	Deoxyribonucleic acid
HACCP	Hazard Analysis and Critical Control Points
HTLV-1	Human T-cell Lymphotropic Virus type-1
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
Il	Interleukin
LcS	*Lactobacillus casei* strain Shirota
NK	Natural Killer
PBMC	Peripheral Blood Mononuclear Cells
URT	upper respiratory tract
URTI	upper respiratory tract infection