**ASSESSMENT REPORT YAKULT®** **10-11-2006**

The undersigned:

1. **Prof R.J. Vonk (chairman)**, Laboratory of Nutrition and Metabolism, University of Groningen
2. **Mrs. E. Kampman PhD**, Division of H uman Nutrition, Wageningen University
3. **F.M. Nagengast PhD**, Department of Gastroenterology, University Medical Centre Nijmegen
4. **Prof. L. de Vuyst**, Research Group of Industrial Microbiology, Vrije Universiteit, Brussel

have been appointed by the Netherlands Nutrition Centre and the Applicant **Yakult Europe BV,** to assess the scientific evidence for the following health benefit:

Product:
**Yakult® fermented milk product**, containing *Lactobacillus casei* strain Shirota (LcS).

Health Benefit:
**Drinking at least one bottle (65 ml) of Yakult® per day**

1. **may improve bowel habit in subjects who are susceptible to constipation**
2. **may support a well-balanced gut microbiota through an increased number of lactobacilli.**

In Dutch:
Consumptie van ten minste één portie (65 ml) Yakult® gefermenteerde melk per dag

1. kan de stoelgang verbeteren bij personen die gevoelig zijn voor constipatie
2. kan bijdragen aan een evenwichtige darmflora door een toename van het aantal lactobacillen.

The decision of the panel reads:
There is **sufficient scientific evidence**, as referred to in the Code of Practice, **for the Health Benefits 1 and 2**, as described above.

**References:**


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A. *What is the panel's judgement of the completeness of the dossier and the interpretation of scientific literature?*

- Probiotics are defined as live microbial food ingredients that are beneficial to health. The most used probiotics are lactobacilli and bifidobacteria (Cummings et al. (2004); Salminen et al. (1998); FAO/WHO (2001)). *Lactobacillus casei* strain Shirota (LcS) – initially classified as *Lactobacillus acidophilus* – was developed in 1935. Yakult® containing LcS bacteria has been introduced on the Dutch market in 1994. The same strain of LcS bacteria that is currently used in the Yakult® product has been tested in several clinical trials.
- The panel appreciates the dossier provided by the Applicant, including an extensive list of references. The quality of the research presented has been judged by the panel according

to the PASSCLAIM criteria (Aggett et al. (2005)). For this reason, the panel considered the results of a large number of earlier trials that were not designed according to present standards as circumstantial evidence. Also, the panel has taken into account that trials that were published in low impact factor journals may provide a lower level of evidence.

- Although many earlier trials were not designed according to present quality standards, the scientific literature on the effects of (Yakult® containing) LcS bacteria has been correctly presented and the panel appreciates the completeness of the dossier.

B. *Are there sufficient data to sustain the viability of Lactobacillus casei strain Shirota in the product matrix of Yakult® fermented milk product?*

The viability of LcS in the matrix of the Yakult® product has been tested by different research groups.

- There are sufficient (confidential) data showing that Yakult® contains at least $6.5x10^9$ CFU live cells up to the end of the expiration date.

C. *Are there sufficient data to sustain the survival of Lactobacillus casei strain Shirota during gastro-intestinal transit?*

- *In-vitro* incubation studies suggest that LcS bacteria survive acid and bile treatments (Gibson et al. 2005). In a predictive model simulating the upper gastrointestinal tract, the survival rate was approximately 10% after gastric passage and 1% after passage of the stomach and the small intestine. Survival in the gastric compartment of the model was 3 times higher in the fermented milk product than in a suspension in non-fermented milk (Marteau et al. 1997; in addition confidential data).
- Survival of LcS in the large intestine after consumption of Yakult® has been shown in a number of human trials. Faecal LcS recovery indicates that the viable cells that reach the large intestine grow and multiply in this area of the intestine (Spanhaak et al. 1998; Yuki et al. 1999; Tanaka & Ohwaki 1993).

- Sufficient data indicate that reasonable numbers of LcS - as present in the matrix of Yakult®- survive during gastro-intestinal transit.
- Survival of LcS bacteria through the intestinal tract does not automatically imply that they are metabolically active, but a number of clinical trials indicate that viable cells grow in the large intestine.
- One study (Spanhaak et al. 1998) indicated that LcS return to baseline levels within two weeks after ceasing consumption of Yakult®.

D. ***Health Benefit 1:*** *Are there sufficient data showing that consumption of Yakult® fermented milk product may improve bowel habit in subjects who are susceptible to constipation? If so,*
   - *What is the minimum effective dose?*
   - *Does this effect on bowel habit imply a relevant health benefit to the target group?*
   - *What is the mechanism of action and are there any possible side effects that may counteract the beneficial effect?*

- Constipation is a widespread phenomenon in western populations (Locke et al. 2000). People suffering from constipation not only may experience abdominal or anal pain, there are indications that they are also at higher risk of developing diseases such as colorectal cancer (Roberts et al. 2003). Constipation is a disorder of motor activity of the large bowel traditionally defined in terms of bowel frequency. Total gut transit time is generally prolonged in constipated subjects. Bowel habit is a useful overall biomarker of gut, especially colonic, function and is usually defined in terms of frequency of defecation, stool consistency and form and stool weight (Cummings et al. 2004; Salminen et al. 1998).
- Two randomised controlled trials in subjects with mild constipation (relatively large groups) showed increased defecation frequency. In the first study the minimum dose of 65 ml (providing ≥ 6.5 x $10^9$ LcS per day) was used (Koebnick et al. 2003). The second study (testing a dosage of ≥ 40 x $10^9$ LcS per day) indicates that fermented milk beverage is more effective in individuals with a clear tendency to constipation. This study also showed improvements in smell of stools and stools were softened (Matsumoto et al. 2006).
- In addition, there are supporting data from earlier trials that investigated the effects of LcS concentrate in constipated subjects, suggesting reduced complaints in over 50% of subjects.
- There are no data on the effect of consuming Yakult® on transit time – a parameter of colonic function that can be objectively measured – in constipated subjects. One randomised controlled trial in healthy non-constipated men (relatively small group) showed no significant effect on transit time (there was a tendency to a decrease), but did show a slight, significant increase in faecal moisture content (Spanhaak et al. 1998).
- The mechanism of action of increased defecation frequency is not yet elucidated, but an increase of peristaltic movements and faecal bulking as a result of an osmotic effect due to production of short chain fatty acids probably may play a role.
- It is not known whether the metabolic activity of lactobacilli may be dependent on the amounts of bacteria ingested. Due to methodological difficulties a dose response relationship cannot be established. The order of magnitude in numbers of bacteria does not differ very much when the intake varies between one or three portions per day. However, studies on the effects of various dosages of LcS on a logarithmic scale have not been performed either.
- There is a long history of safe use of LcS in humans. Besides there is no evidence that probiotic lactobacilli pose any greater risk of infection than do commensal strains. The available data indicate that consumption of LcS in the usual dosage presents a negligible risk to consumers, including immunocompromised hosts (Asahara et al. 2003; Borriello et al. 2003; Marteau & Shanahan, 2003).
- Yakult® is positioned by the Applicant as a product which should be used in addition to the habitual diet. Assuming that people use the recommended amount of Yakult® (65 ml) in addition to the normal daily diet, it is anticipated that the daily intake of nutrients and energy slightly increases with about 2% on energy basis (1.4% with Yakult® Light). In case subjects exchange a glass of skimmed milk (150 ml) for Yakult® (65 ml), calcium intake will decrease by about 170 mg per day.

Considering that

- Two randomised controlled trials in subjects with mild constipation – together with supportive data from earlier trials - show that consumption of Yakult® increases defecation frequency and results in softer stools;
- Although there are no data on dose-response, it is reasonable to assume that a minimum dose of 65 ml may increase defecation frequency among those susceptible to constipation;
- Although the effect on transit time is not clear (has not been measured in constipated subjects),

**It can be concluded that in subjects who are susceptible to constipation an increase in defecation frequency with consumption of at least one bottle of Yakult® may imply a potential health benefit.**
**Any major side effects are not likely to occur as supported by data from clinical trials (lasting up to 4 weeks).**
**It is not clear whether subjects with normal bowel habit may experience any effect on bowel function.**

E. ***Health Benefit 2:*** *Are there sufficient data showing that consumption of Yakult® fermented milk increases the number of lactobacilli in human faeces? If so,*
   - *What is the minimum effective dose?*
   - *Is it reasonable to assume that an increase in these bacterial counts may support a well-balanced gut microbiota?*
   - *Does this imply a relevant health benefit?*
   - *What is the mechanism of action and are there any possible side effects that may counteract the beneficial effect?*

- A broad distinction can be made into 3 categories of colonic bacteria: a) pathogens such as clostridia; b) commensal bacteria such as bacteroides that can have both positive and negative effects; and c) lactobacilli and bifidobacteria that are assumed to have beneficial effects on colonic function. There is no generally accepted definition of a well-balanced gut microbiota available. However, a large number of human trials indicate that consumption of probiotic bacteria induces a shift in the composition of the gut microbiota towards a larger percentage of beneficial bacteria (Mitsuoka, 1990; Hopkins et al. 2001; Cummings et al. 2004; Salminen et al. 1998).
- Three controlled trials – 2 studies in healthy adult males, 1 study in (mainly female) adult volunteers with mild constipation - showed an increase in lactobacillus counts (mainly strain Shirota) by 1 to 3 log units CFU/g faecal material. After cessation of Yakult® intake the numbers of lactobacilli decreased, indicating that the resident population is still present, and is not replaced by the probiotic strain. The background level of lactobacilli in these studies varied by 2 log units, reflecting cultural and/or geographical differences in microbial composition (Matsumoto et al. 2006); Spanhaak et al. 1998; Tanaka & Ohwaki 1993). Because numbers of lactobacilli are only a very small percentage (< 1%), it should be realised that a significant effect on the percentage of total gut microbiota usually cannot be found.
  Although there are indications for favourable changes in other microbial species besides lactobacilli, the results are not very consistent.
- Like bifidobacteria, lactobacilli are suggested to have an inhibitory effect on other (possibly pathogen) bacteria species, possibly related to the production of lactic acid and short chain fatty acids and consequently a lowering of local gut pH to levels below that at which other bacteria are no longer able to grow. However, also other mechanisms of inhibition – such as competition for nutrients and adhesion sites on the gut wall - may also be involved (Gibson & Wang 1994; Tuohy et al. 2003).
- Clinical trials by De Preter et al. (2004) suggest that consumption of Yakult® may suppress the generation and accumulation of potentially toxic fermentation metabolites ($NH_3$ and p-cresol) in the large intestine. Also, a lower enzyme activity of β-glucuronidase and β-glucosidase has been reported by Spanhaak et al. (1998), which may be indicative of reducing potentially harmful effects of the gut microbiota. An increased concentration of butyric acid – which is considered to be beneficial although the long term health effects are still uncertain - has not been unequivocally shown.

- As outlined in Section D, a dose response relationship cannot be established and major side effects are not expected.

Considering that

- An increase in faecal numbers of lactobacilli with consumption of Yakult® has been sufficiently shown;
- Although there are still uncertainties with respect to the consequences of increased numbers of lactobacilli for long term health in the population at large;
- There is circumstantial evidence that increased numbers of lactobacilli may lead to a reduction of potentially harmful compounds. These effects can be indirectly related to bowel habit; with faster gastrointestinal transit there is less opportunity for generating harmful compounds. It should be noted that the experimental data are based on measurements in faeces and urine. In order to allow for more definite conclusions, reduction of the formation of harmful compounds should be investigated in more detail,

**It is reasonable to assume that an increase in lactobacilli counts with consumption of Yakult® may support a well-balanced gut microbiota.**
**Sustained intake of Yakult® is necessary to keep this beneficial effect.**

This decision was reached and undersigned by,
The members of the panel:

Prof R.J. Vonk (chairman)

Place:_____
Date:_____

F.M. Nagengast PhD

Place:_____
Date:_____

Mrs. E. Kampman PhD

Place:_____
Date:_____

Prof. L. de Vuyst

Place:_____
Date:_____