

## Reviewer's report

**Title:** Four-week short chain fructo-oligosaccharides ingestion leads to an increase in fecal bifidobacteria and cholesterol excretion in healthy elderly volunteers

**Version:** 1 **Date:** 1 October 2007

**Reviewer:** Randal Buddington

### Reviewer's report:

#### General

There is a well established, and accepted, relationship between supplementing the diet with prebiotics and increasing the proportion of the GI bacteria represented by species considered to provide a diversity of health benefits. The senior author (Y Bouhnik) has been an author for several papers that describe and contribute to our understanding of how the prebiotic scFOS increases fecal bifidobacteria in human subjects. The present contribution describes a study that examined if and how supplementing the diet of 12 elderly subjects changes fecal bacterial and chemical composition and stool characteristics and patterns. Since elderly subjects exhibit declines in health promoting bacteria and are at greater risk of GI disturbances, the contribution addresses an issue of relevance to health, particularly with the increasing percentage of the population represented by the elderly.

The manuscript will require editing to correct grammar and spelling. The following comments are provided to assist the authors in addressing issues that some readers will find uncertain or controversial.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The most pressing concern is that the study is not as novel as the authors contend. There have been several previous studies that have examined if and how prebiotics, including scFOS, influence the fecal bacteria of elderly humans. For example, Mitsuoka T, Hidaka H, Eida T. Effect of fructo-oligosaccharides on intestinal microflora. *Nahrung*. 1987;31(5-6):427-36. And there is a review available (Hamilton-Miller JM. Probiotics and prebiotics in the elderly. *Postgrad Med J*. 2004 Aug;80(946):447-51). Additional papers have described similar responses in elderly dogs (Grieshop et al., Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. *Arch Anim Nutr*. 2004 Dec;58(6):483-93).

The authors will need to expand their review of the literature as others have previously examined if prebiotics influence the fecal bacteria in elderly subjects. Some readers will be aware of these references. The authors will also need to

inform readers how the present contribution expands the literature by providing new information.

2. Methods: Some of the methods are not adequately described.

a. Were the radio-opaque pellets that were fed a mixture of different shapes, or was a different shape fed each day? Is there any evidence that the pellets traverse the GI tract at the same rate as digesta?

b. How were the bifidobacteria identified on the plates? Because the Beeren's medium used is a semi-selective agar, other bacterial groups will grow. Unless the authors confirmed the identities of representative colonies growing on the plates (e.g., by the fructose-6-phosphate phosphoketolase activity), the reported counts are not necessarily bifidobacteria and may be misleading, or at the least inaccurate.

c. Were the stools diluted before measuring pH, or was the pH probe placed into the solid stool?

d. Were the methods used for bile acids and neutral sterols validated? Was the percent recovery determined to ensure readers the method is appropriate? Since a modification of a previous method was used, readers (and reviewers) will want to know if this improved the analysis, and if so how.

e. Log bacterial counts are generally transformed prior to analysis. The authors may want to consult a statistician about the most appropriate method to analyze their data.

3. Results

a. In light of the above concern of bifidobacteria identification, this reviewer has concerns about whether the data are 'real'

b. The authors may want to consider another approach for analysis of the data. Specifically, since each subject effectively serves as their own control, the authors may want to consider expressing fecal characteristics as a percentage or ratio of the value measured during the initial period before the scFOS supplement. This accounts for individual variation and may reveal differences that are obscured because of individual variation in absolute values. It is possible that differences will be detected where none are now evident.

c. Similarly, the authors could prepare ratios of apparent 'bifidobacteria' relative to clostridia and enterics, or the percentage of anaerobic bacteria represented by the different bacterial groups.

d. Figure 1 should be redone such that each of the four measured parameters are presented as 3 bars basal, scFOS, follow-up. This will make it easier for readers to immediately see if there is an influence.

4. Discussion

a. Can the authors speculate on the mechanism responsible for the increase in fecal cholesterol?

b. Is it possible that the bacterial assemblages in the elderly respond slower than

those of younger individuals? If so, the 4 week period might not have been long enough to alter both the species composition and metabolic activities.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

1. The authors may want to discuss how various doses of scFOS have been used and the dose-response relations. Is it possible that a larger dose is needed for elderly subjects? The authors do raise this question, but leave readers wondering. Also, the authors mention a dose-response relationship noted among animals models for poorly digested carbohydrates.

**What next?:** Accept after minor essential revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.