

## 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:** 4 September 2007

**Reviewer:** Kevin Whelan

### Reviewer's report:

General:

This is an interesting and carefully conducted investigation of the impact of scFOS on faecal microbiota, pH and sterol concentrations. It appears to be a well conducted study with appropriately measured outcomes. Although not entirely novel in terms of the impact of scFOS on the faecal bifidobacteria, this study is novel in terms of its age group (older people), impact upon sterol metabolism and impact upon other microbiota. The authors should be congratulated on some of their extremely novel findings relating to impact upon faecal clostridia.

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

Results and discussion – the major improvement required from this manuscript is extensive discussion of the remarkable effects on faecal clostridia. The results show that scFOS supplementation increases clostridia and withdrawal of scFOS then increases them even further – the authors are truly the first group to have demonstrated such effects. Although these changes are mentioned in the results they are not mentioned in the discussion. These are major observations and warrant extensive discussion, particular due to the age related changes in microbiota and the increased risk of *C. difficile* colonisation in older people. Perhaps supplementation may be negative in older people?? Older people may be more frequent antibiotic users – and certainly in vitro studies (MacFarlane) have shown that FOS supplementation in the presence of antibiotics actually increases clostridia. In view of the effect of scFOS on clostridia there should be significant discussion of this finding and the title of the manuscript should also probably reflect this.

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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)

Introduction - you should make reference to the studies already done of prebiotics in older people in the introduction – although you quote Bartosch there are others (e.g. Bunout, JPEN, 2002 – although didn't measure microbiota).

Methods – how (and where from) were subjects recruited.

Methods – please justify why scFOS was chosen rather than inulin (for example)

Methods – it should be made even clearer that OFTT was measured at the end of each of the feeding periods.

Methods – please provide brief details of how the pellets were measured in faeces (presumably X-ray).

Methods – please clarify – were stools frozen prior to culturing or were they cultured fresh? If they were cultured following freezing then the limitations of this should be acknowledged and the interpretation of the results should be amended accordingly.

Methods – page 7 – “outnumber” – is this the correct word?

Methods – were samples diluted in buffer prior to pH measurement? If so details of the buffer should be provided.

Data analysis – details of data analysis of bile acids etc should also be provided (2 way ANOVA presumably).

Results - first line – refers to “control” – please be consistent with terminology e.g. ‘baseline’ or ‘follow-up’.

Discussion - the discussion on page 13 includes the following sentences which seem to be contradictory:

“microbial transformation of cholesterol into coprostanol was not influenced by scFOS in our study”

“ingestion of low dose of scFOS .....prevented microbial conversion of cholesterol to....coprostanol.....”

Discussion - the discussion regarding increases in faecal cholesterol are reportedly due to the impact of the microbiota in blocking cholesterol conversion to coprostanol. However, wouldn't this result in a reduction in coprostanol concentrations (which didn't occur)? Is it at all possible that the increase in cholesterol concentrations may be due in part to the scFOS affecting cholesterol absorption in the gut?

General – the manuscript is generally well written but would benefit from review by a scientist with English as a first language.

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Discretionary Revisions (which the author can choose to ignore):

Methods – you ask subjects to exclude FOS containing foods from their diet. There are number of issues with this:

-firstly the largest source of FOS in the diet is from wheat – was this excluded – if so then, as wheat is so ubiquitous in Western diet this would have made a significant change to the overall dietary and nutrient intake.

-Although asparagus, artichoke etc contain high levels of FOS they are consumed in such low quantities to contribute negligible amounts of FOS to the diet (see Moshfegh) – perhaps their exclusion was unnecessary.

Methods – the limitations of culture-based enumeration of the microbiota should be acknowledged (perhaps in the discussion).

Methods – you say that subjects recorded frequency and consistency of stools and a cut-off for diarrhoea was defined. These results are not reported in the text. Also, consistency is notoriously subjective for people to record. How was stool consistency rating improved (e.g. stool charts??).

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

**Level of interest:** An article of importance in its field

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

**Statistical review:** No, the manuscript does not need to be seen by a statistician.