Author’s response to reviews

Title: A yeast fermentate improves gastrointestinal discomfort and constipation by modulation of the gut microbiome: results from a randomized double-blind placebo-controlled pilot trial.

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Author’s response to reviews:

Dear Editor(s),

We would like to thank you for having accepted for potential publication our research article “A yeast fermentate improves gastrointestinal discomfort and constipation by modulation of the gut microbiome: results from a randomized double-blind placebo controlled trial”.

After careful examination of the reviewers’ suggestions we have changed the manuscript accordingly. Hereby we respond to each of the raised questions:

Gordon Howarth (Reviewer 1):

1. Cause and effect are difficult to dissect out in microbiota studies. Some discussion of alternative explanations should be included.

We agree. Previous in vitro studies have shown that EpiCor increases butyrate levels, and butyrate is involved in intestinal motility. The possible indirect effect via butyrate production has
now been included in the discussion, although from this study we have no evidences that this could be a possible mechanism of action (Lines 658-663, Page 30).

2. p-values (or similar) should be included in the abstract to give some indication of magnitude of effect.

We agree. It is now included in the abstract for the primary objectives (Lines 41-47, Pages 2 and 3).

3. There are some minor grammar issues (eg misspellings such as 'enrol'). The Discussion is a little laboured and verbose but overall the critique of the work is described well.

We have used the American automatic corrector for word, and according to the grammatical rules, ‘enroll’ is used inside America and ‘enrol’ outside the U.S. Although the manuscript has been written by a non-native English speaker (the first author), the second author (which is a native English speaker (from the U.S.)) has revised the manuscript. Nevertheless, we took this suggestion into account and have replaced ‘enroll’ by ‘enrol’ and ‘enrollment’ by ‘enrolment’. Note that ‘enrolling’ and ‘enrolled’ are nevertheless spelled with two l’s. However, if this (or other grammatical aspects) are still an issue, a second round of revision by the second author can be done.

4. Figures need work as they are very blurred.

We have saved the figures in a different format. We hope it is better now. If the problem still persists please do not hesitate to contact us. We can still try to improve them.

Gerard Mullin (Reviewer 2)

1. Line 102. Is there evidence in this paper that gut barrier function is altered by EpiCor fermentate? If not, please rephrase as the sentence suggests so. Reference 24 used to support the statement does not demonstrate barrier function is altered by this fermentate.

We believe the reviewer is referring to reference 22 and not 24. Although the mentioned protective effect on barrier function has been experimentally demonstrated by measuring LPS in the serum of animals in a heat-stress model, it has only been shown in rats (reference 22). Therefore we agree that it does not apply to the sentence where it is imbedded, which is referring
to humans. For a matter of scientific rigor we have deleted ‘gut barrier function’ from the sentence and consequently we have also deleted reference 22 (Line 103, page 5).

2. Line 138. The use of probiotics, prebiotics and synbiotics are excluded for 30-days. There are many studies shown the ‘washout” time for probiotics can take weeks after cessation. Perhaps address in the methods or discussion.

While this may be true from some probiotics, usually, if not taken on a daily basis, probiotics tend to be washed out from the organism quite rapidly, as they are outcompeted by the commensal microbiome. In some studies similar to ours, this exclusion criteria is only even required at screening (see for example Buchwald-Werner S, Fujii H, Reule C, Schoen C. Perilla extract improves gastrointestinal discomfort in a randomized placebo controlled double blind human pilot study. BMC Complement Altern Med. 2014;14:173.). We have opted for 30 days to account for most long-lasting effects, and also because it was a compromise between being able to recruit subjects that would meet the numerous inclusion/exclusion criteria and still being able to have a solid scientific output. Constipated subjects that are currently not taking any kind of medication or supplementation are very difficult to find. Therefore, upon suggestion from the D.R.U.G. Unit, which was responsible for recruiting and conducting the study, we have agreed that 30 days prior to study (which was actually longer if one considers the two-week run-in phase plus allocation and appointment to baseline visit) to be a reasonable time frame for wash-out.

3. Line 142. The 5-point GI symptom scale used to assess impact was this a validated tool such as the IBSSS? Reference 14 is cited.

The citation is correct. Our study has been rather based on the following publication from this same journal: Buchwald-Werner S, Fujii H, Reule C, Schoen C. Perilla extract improves gastrointestinal discomfort in a randomized placebo controlled double blind human pilot study. BMC Complement Altern Med. 2014;14:173. Therefore, it has been described and used in similar studies. It is actually a very useful tool, more than other validated questionnaires which are quite long (> 10 questions) and that are only used retrospectively. The advantage of this diary it’s the fact that the subjects only have to grade 5 questions, which is considerably less of a burden. The fact that it is filled in every day makes it also more objective, certainly for clear physical parameters such as feeling of fulness, bloating/distension, GI rumbling, etc. It is our conviction that for such quite objective parameters a diary is more useful and accurate than a retrospective instrument only filled in over the past 2 or 3 weeks. We now refer to this study as being the source of this instrument in Lines 239-240, Page 12, in a clearer manner.
4. Line 161. Are any of the university-based authors have commercial ties to the sponsors of the study (Prodigest BVBA Belgium, Embria USA)?

Yes, this information is now given in the section ‘Competing interests’ (Lines 714-715, Page 32).

5. Lines 184-186. The sponsor labelled all bottles but who conducted the randomization using randomization.com? According to line 226, the sponsor labelled all bottles AND conducted the randomization.

That is true. However, both randomization and labelling of the bottles were done by a staff member of ProDigest that had no direct participation in the study (neither study design, sample collection/analysis or data analysis). Moreover, the encapsulation was identical (and opaque) for both products, and Embria just labelled the bottles as A or B before sending them to ProDigest. Therefore, upon arrival to ProDigest, it was not possible to distinguish the two products and so the randomization list was done for ‘A’ and ‘B’ and the bottles labeled with the allocation number only (so that the subjects would not even know if they were taking an ‘A’ or a ‘B’. Therefore, we are quite confident on the blinding procedure, as only Embria knew what was standing for ‘A’ and ‘B’. This is perhaps now better explained in Lines 181-187, Pages 9-10 and further confirmed in Line 213, Page 11 and Lines 227-231, Page 11.

6. Line 372. EpiCor and placebo did not differ (significantly) for its effects on symptoms on those with severe global symptomatology but did show differences in subgroups. Could the placebo have altered GI symptomatology? It was shown to increase the F/B ratio (line 416, Figures 6a, a*) which portends to worse symptoms and has FODMAP subcomponents (oligosaccharides).

It is indeed a possibility. However, as stated on Lines 178-180, Page 9, maltodextrin is the most commonly used placebo in dietary studies evaluating gut microbiota and intestinal well-being because it is easily digested and rapidly absorbed as glucose and has no anticipated effect on colonic fermentation. However, we cannot exclude with certainty the possibility that maltodextrin is altering the microbiome composition and leading the observed increase in F/B ratio. However, if this increase in F/B ratio is expected to worsen symptoms as suggested by the reviewer (as a FODMAP) then we would expect for the GI symptoms reported in the diary to worsen and not to improve (within the placebo group). We actually believe that the placebo effect reported in the questionnaires is more a self-reported improvement (a real placebo effect) rather than a translation from a physiological outcome. The possibility that subjects may have altered their dietary habits which could have led to the differences observed within the placebo-treated groups in terms of microbial composition is discussed on Lines 430-433, Page 20.
7. Lines 560-1. Serotonin and gastric are referenced to influence neurogastrointestinal function. Consider peptide YY and GLP-2 which are enteric hormones which alter motility and are influenced by SCFA and fermentates.

We agree. We have referred to the two hormones on Lines 563-564, Page 26. Of note it is possible that the reviewer refers to GLP-1 and not GLP-2.

8. Lines 625-6. "For instance, a significant increase in Akkermansia muciniphila was observed within this subgroup." Please define "This subgroup" in the sentence.

It has been done (now Line 629, Page 28).

We believe we have addressed all raised questions conveniently. However, if some issues still persist, please do not hesitate to contact us. Once more we thank you for accepting our manuscript for potential publication. Of note, some of us will be on holidays in the coming next 2 to 3 weeks, which may delay answers. We will try to regularly check the email, though.

Hoping to deserve your best appreciation.

Yours sincerely,

Sam Possemiers