**Reviewer’s report**

**Title:** Randomised Clinical Trial: Effect of low-FODMAP rye bread versus regular rye bread on the intestinal microbiota of irritable bowel syndrome patients: Association with individual symptom variation

**Version:** 0  **Date:** 30 Oct 2018

**Reviewer:** Alessia Visconti

**Reviewer’s report:**

The manuscript describes a cross-over intervention study aiming at understanding whether the increase of fibre intake in IBS patients via low-FODMAP and regular rye bread could alter their gut microbiome composition and improve their symptoms. As expected, they failed to observe a massive shift in the microbiota profiles. However, they confirmed observations collected during other metagenomics studies (including one authored by the same group) which suggest that the baseline intestinal microbial composition influences the efficacy of dietary interventions, and which this Reviewer finds the most interesting message of the study.

The main concern of this Reviewer is the definition of responders vs non-responders to the intervention. In fact, the authors hypothesise that the low-FODMAP bread would improve IBS symptoms (considering as responders those that showed a reduction of IBS-SSS score of at least 50 points and/or symptom of pains more than 10mm at VAS), while the regular rye bread would worsen IBS symptoms (considering as responders those that showed an increase of IBS-SSS score of at least 50 points and/or symptom of pains more than 10mm at VAS). Can the authors clarify why they decided to use different criteria to define responders/ non-responders in the two arms of the study? How would the results (especially those on microbiota composition differences at baseline) change if the same criterium (let say, that used for the low-FODMAP bread) would be used for both arms? Did any individual in the low-FODMAP arm showed a major worsening in their symptoms (analogous to those measured in the individuals in the regular rye bread)? If yes, did they show significant microbiota difference from individuals without these symptom changes (at baseline and after the intervention)?

In a previous study which uses the same subjects, the authors have already shown that while some signs of IBS where milder on the low-FODMAP rye bread, no differences were detected in IBS symptoms or quality of life and that both bread were able to increase the fibre intake [doi.org : 10.1111/apt.13726]. This reviewer find that similar results are shown it this secondary study, and wonder whether the small shifts in bacterial composition are due to the intake of either or of the breads, or simply by an increase on dietary fibre (given the baseline composition, and the large adjusted P value observed). In fact, two messages seems to be lurking from the Discussions. One says that there is no differences between breads in term of microbiota and that fibre are responsible for the observed changes (lines 290-297 and 317-319), the other that low-FODMAP rye bread is beneficial (lines 303-306), and of which this Reviewer is not fully convinced. Can the authors comment on this?
Minor comments:

1. The authors state that α-diversity and richness were not affected by the intervention. However, this Reviewer wonders if the baseline α-diversity/richness could predict whether a subject is a responder or a non-responder to either of the breads.

2. The study includes mostly female (94%). Could the authors comment whether this makes their study less generalizable and whether including only two males generate "noise", and different results would be reached if males are dropped out and/or if sex is included as a confounder?

3. Study participants show a very wide range of symptoms severity score. Have the authors investigated whether this score is influenced by the baseline microbiota composition, and whether this could predict the response to either of the two breads (including microbiota shifts)?

4. Can the authors include the results of the PCoA using the Bray-Curtis dissimilarity as Supplementary Material?

5. Lines 119 and 120 describe the two bread as both "sourdough bread", while Table 1 describes them as "sourdough bread" and "Yeast-fermented bread". Can the authors confirm that they are both sourdough, and clarify which bread is described by the two columns in Table 1?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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