Author’s response to reviews

Title: The effects of synbiotic supplementation on hormonal status, biomarkers of inflammation and oxidative stress in subjects with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial

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Author’s response to reviews:

Dear Dr Ziemnicka,

Thank you very much for your letter informing us of your decision regarding the manuscript no. BEND-D-18-00022R1 entitled "The effects of synbiotic supplementation on hormonal status, biomarkers of inflammation and oxidative stress in subjects with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial". The manuscript has been revised according to the referees’ comments. Responses to the reviewers’ comments have been provided below. Thank you so much in advance.

Yours Sincerely,

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First of all, we would like to thank the reviewer for the great comments to improve the quality of our manuscript. We tried our best to revise the manuscript according to the reviewer's comments. We are happy to hear that your concerns have been addressed.

Zuleyha Karaca (Reviewer 1):

In this paper, Nasri et al investigated the effects of synbiotic supplementation on hormonal status, biomarkers of inflammation and oxidative stress in subjects with PCOS. The paper seems interesting however I have some concerns;

1. The time period of 12 weeks for treatment is too short to evaluate the effects on hormonal parameters and FGS. How did the authors decide for this time, please explain and discuss the limitations.

Authors: Thank you for this important comment. This point added to Limitation section:

“Duration of the treatment was selected based on observed beneficial effects of probiotic supplementation on metabolic profiles in women with PCOS [16]” (Lines 120-1).

Reference


“However, duration of the treatment was too short to evaluate the effects of synbiotic supplementation on hormonal parameters and mFG scores; we believe that future studies with cross-over design and longer duration of the intervention are needed to confirm the validity of our findings” (Lines 307-10).
2. In the abstract the number of controls is 35 but it is stated as 30 in the paper.

Authors: Thank you. I apologize for this mistake. This point corrected in the revised version:

“Subjects were randomly assigned into two groups to take either synbiotic (n=30) or placebo (n=30) for 12 weeks” (Line 44).

3. The authors state that the data regarding synbiotic supplementation is scarce but they haven't discussed the previous data if any.

Authors: Thank you. I apologize for this confusion. This point corrected in the revised version as follows:

“To our knowledge, no reports are available indicating the effects of synbiotic supplementation on hormonal status, biomarkers of inflammation and oxidative stress in subjects with polycystic ovary syndrome (PCOS)” (Lines 38-40 and 89-91).

4. In the results section; the effects of treatment on measured parameters are given. Please state which is before and after treatment.

Authors: Thank you. This point clarified in the revised version:

“After the 12-week intervention, compared with the placebo, synbiotic supplementation significantly increased serum SHBG (changes from baseline in synbiotic group: +19.8±47.3 vs. in placebo group: +0.5±5.4 nmol/L, p=0.01), plasma NO (changes from baseline in synbiotic group: +5.5±4.8 vs. in placebo group: +0.3±9.1 µmol/L, p=0.006), and decreased mF-G scores (changes from baseline in synbiotic group: -1.3±2.5 vs. in placebo group: -0.1±0.5, p=0.01), FAI (changes from baseline in synbiotic group: -0.12±0.29 vs. in placebo group: -0.01±0.08, p=0.01) and serum hs-CRP (changes from baseline in synbiotic group: -950.0±2246.6 vs. in placebo group: +335.3±2466.9 ng/mL, p=0.02) (Table 2). In addition, compared with the placebo, synbiotic supplementation resulted in a significant reduction in serum insulin levels (changes from baseline in synbiotic group: -1.6±2.9 vs. in placebo group: +0.4±2.3 µIU/mL, p=0.003), HOMA-IR (changes from baseline in synbiotic group: -0.4±0.7 vs. in placebo group: +0.1±0.5, p=0.003). A trend toward a greater decrease in total testosterone (changes from baseline in synbiotic group: -0.4 vs. in placebo group: -0.1 ng/mL, p=0.09) and plasma MDA concentrations
(changes from baseline in synbiotic group: -0.2±0.1 vs. in placebo group: +0.5±1.4 µmol/L, p=0.05) was observed in synbiotic group compared with placebo group. We did not observe any significant effect of synbiotic supplementation on other hormonal status and biomarkers of oxidative stress” (Lines 48-57 and 205-21).

5. The SDs of some parameters in the tables is too high. Please check if it is appropriate to give mean value.

Authors: Thank you for this important comment. This point clarified in the revised file:

“Outcome log-transformation was used if model residual has non-normal distribution (hs-CRP, MDA, SHBG and FAI)” (Lines 182-3).

“Furthermore, the high standard deviations (SDs) of dependent variables in some cases might make the interpretation of our findings difficult. Such high SDs might be explained by the small number of participants in the study, which was a limitation in our study” (Lines 310-3).

6. How do the author interprete the decrement of FGS by 1.3 in 3 month’s time. The change is minimal clinically although statically significant and the time period is very short. Do the authors expect further decrease in FGS by time?

Authors: Thank you. I agree with you. This point added to the revised version:

“However, observed reduction at mFG scores after 12 weeks was statistically significant, it was clinically low. Long-term interventions and higher dosage of probiotic and inulin might result in greater changes in mFG scores” (Lines 234-6).

7. Discuss the paper by Shoaei T in 2015 regarding probiotic supplementation in PCOS.

Authors: Thank you. This paper discussed in Discussion section:

“Shoaei et al.[24] also indicated that probiotic supplementation for 12 weeks to women with PCOS significantly decreased fasting glucose and insulin concentrations” (Lines 247-9).
Reference


Michael O'Reilly (Reviewer 2):

Nasri and colleagues present a well-designed study on the effects of synbiotic supplementation compared to placebo in an Iranian PCOS cohort. The study is well designed and suitable for publication once some essential revisions are made.

Authors: Thank you.

1. The principal limitation of this study is the lack of a crossover element to supplementation. This means that two heterogeneous groups are being compared, despite the careful matching that was conducted. This needs to be acknowledged in the discussion.

Authors: Thank you for this valuable suggestion. This point addressed in Limitations section:

“However, duration of the treatment was too short to evaluate the effects of synbiotic supplementation on hormonal parameters and mFG scores; we believe that future studies with cross-over design and longer duration of the intervention are needed to confirm the validity of our findings” (Lines 307-10).

2. Why was insulin not measured before and after in each treatment arm? This may provide some insights as to whether all the observed effects are secondary to insulin resistance.

Authors: Thank you. Data on insulin levels and HOMA-IR added to the revised version:

“In addition, compared with the placebo, synbiotic supplementation resulted in a significant reduction in serum insulin levels (changes from baseline in synbiotic group: -1.6±2.9 vs. in placebo group: +0.4±2.3 µIU/mL, p=0.003), HOMA-IR (changes from baseline in synbiotic group: -0.4±0.7 vs. in placebo group: +0.1±0.5, p=0.003)” (Lines 212-5).
3. There is not enough in the discussion on PCOS-specific effects. Most of the comparison is made in studies with T2DM or GDM. What is unique about PCOS that makes this a suitable group for the intervention? Insulin resistance alone is not sufficient to discuss in this regard, and greater exploration of androgen excess pathology must be alluded to in discussion.

Authors: Thank you. This point addressed in the revised version:

“Hyperinsulinemia and insulin resistance in women with PCOS directly stimulate ovarian steroidogenesis by acting on thecal cell proliferation and increasing secretion of androgens mediated by luteinizing hormone (LH), increased gene expression of cytochrome P450 and insulin-like growth factor 1 receptor [30]. In addition, androgens may regulate follicular atresia [31]. It was also reported that increased testosterone levels increase somatic cell atresia in rat ovaries [32]. Furthermore, hyperandrogenemia can induce inflammation in women with PCOS [33]. Therefore, synbiotic intake due to its useful effects on insulin resistance may be useful to control clinical and metabolic symptoms” (Lines 254-61).