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Response to Reviewers

We greatly appreciate all of the valuable suggestions and comments from the reviewers. We have revised the manuscript based on these constructive suggestions and hope the point-by point responses would fully address your concerns.

Reviewer 1.
The impact of antenatal nutritional interventions on the development of the immune system is a fascinating topic that is of much interest to researchers currently. Prebiotic fibres are an enticing biologic substrate, and are becoming increasingly widely studied in relation to alter their immunologic effects.

The presented work focuses on antenatal supplementation of a fibre, short-chain fructooligosaccharide (FOS), and a toxin, mycotoxin deoxynivalenol (DON), and their subsequent effect on offspring in a swine model. This is an ambitious project, and I commend the authors for the major effort that certainly was undertaken to perform this project in a large animal model. However, there are substantial concerns regarding the scientific merits of this project, most concerningly relating to the experimental design.

Thank you for your encouraging comment. Indeed, it was an ambitious and time-consuming project.

Major concerns

1) The experimental design as presented in figure 1 and described in the methods does not seem to present adequate control groups for comparisons. Specifically two issues standout, namely comparing non-gavaged animals to gavaged animals, and the comparison of the DON and FOS groups.

Ultimately, this project attempts to compare a number of different relationships. Below are, in my view, these relationships, and individual issues with their appropriateness:

a) Prior to birth, the effect of FOS supplementation and DON exposure on sows compared to a control diet.

There is no explanation for why FOS and DON should be directly compared. These are two interventions, intending to produce disparate results, through presumably different mechanisms. Both may be appropriately compared to a control diet, but not to each other.

We agree with this comment. Accordingly, we modified Figures 2 and 3 and the text in the paragraph 3.1 and paragraph 3.2 of the Results.

b) Comparing DON-gavaged piglets to sham-gavaged piglets (CTRL-).

These comparisons are appropriate with an adequate control group.

Indeed, there is no bias due to the gavage with excipient (CTRL-) or DON, as all animals of these two groups were force-fed at the same time from 1 to 8 days of age.

c) Comparing perinatal FOS-supplementation to sham-gavaged piglets (CTRL-).

Gavage produces significant stress to animals. It would therefore be difficult to note whether difference between these two groups are due to FOS, or to the stress of gavage. In short, the two groups were not treated similarly outside of the FOS supplementation, and therefore inferences regarding the impact of the FOS cannot be made.

d) Comparing perinatal DON-exposure to sham-gavaged piglets (CTRL-).

Gavage produces significant stress to animals. It would therefore be difficult to note whether difference between these two groups are due to DON, or to the stress of gavage. In short, the two groups were not treated similarly outside of the DON supplementation, and therefore inferences regarding the impact of the DON cannot be made.

e) Comparing perinatal DON-exposure to FOS-supplementation.

This comparison places two interventions, both of which have unknown effect on the measured variables, in competition with one another. Any differences therefore, cannot be ascribed to either treatment.

I feel that the utility of this work would be greatly enhanced by the addition of a true control group for the PREB and DON groups, one that does not gavage it's piglets.

Response to c) d) and e) comments:
The oro-gastric gavage may produce a significant stress to the animals, even though it was performed by very experienced and qualified staff. A group of animals without any gavage would have been the best animal control group to be compared with PREB and DON groups. Nevertheless, we would like to stress the need to strike a balance between the practical aspects of our study, *i.e.* . to perform an experiment with a manageable number of animals considering the ethical rules and limiting as much as possible the number of experimental animals, the number of time-points we have done and the necessity to compare sham-gavaged and DON-gavaged animals within litters. Therefore, the findings of this study should be interpreted in light of its inherent limitations. We have now pointed these limitations in the discussion (lines 625-629).

2) Caloric intake is often a confounding factor in the studies of this type, and must be addressed. Was any attempt made to assess the caloric intake of the sows? Given the differences in sow performance noted in figure 1, have the authors considered that overall caloric intake may be responsible for some of the subsequent findings, rather than direct effects of the fibre.

Response:
All sow diets were formulated to provide the same energy. As there were no differences in daily dietary intake between CTRL and PREB sows in one hand, and CTRL and DON sows in the other hand, the difference in the back fat thickness between CTRL and PREB sows after farrowing may be explained by a direct prebiotic effect on sow metabolism (as noted in the discussion), but is not the result of any difference in energy intake.

3) The statistical analysis of this paper may be inappropriate. It is stated that data were assessed for normality; however, most groups identified would have 4 or fewer samples. Demonstrating

deviations from normality with this limited a sample size is very difficult. Therefore, the majority of the analysis should be done using non-parametric tests. Additionally, the use of 'trends' or representing $p<0.1$ as near significant is worrisome, especially given the massive number of comparison performed in this work.

Response:
We agree with the reviewer comment. We have provided additional details in the Material and method section in « Statistical analysis » paragraph (lines 210-222). Use of parametric tests only concerned data on sow and piglet performances (with low inter-individual variability and higher animal numbers per group) while non-parametric tests were applied on data of flow cytometry, qRT-PCR and ELISA assays (greater variability and smaller sample number per group). However, we propose to keep the information on tendency when it occurred. As individual data points are now presented, the reader can clearly appreciate the effects.

Minor concerns

1) Non-significant relationships should not have '#' or '*' above them in figures, even when those are defined in the figure legend. At a glance these are very misleading. Further, the definitions of these symbols should be consistent between figures (sometimes # means <0.1, other times <0.01)

Response:
We are sorry for these errors. We have thoroughly revised all figures and the mistakes have been corrected.

2) SEM is the measure of dispersion presented. This is rarely the appropriate choice, and SD may be more appropriate.

Response:
We make changes in the revised version. Means ± SEM are used for normally distributed data (sow and piglet performances) while means ± SD are used for qRT-PCR and ELISA data.

3) The figures all present means with SEM bars. Given the low number of data points (4 or less for each comparison) it may be helpful to present individual data points, alongside the mean and measure of dispersion for the reader.

Response:
We agree with this relevant comment. Figures concerning qRT-PCR and ELISA data have been re-drawn presenting individual data points of qRT-PCR and ELISA data (Figures 5, 6, 8 and 9).

4) There are numerous small grammatical errors in the work.

Response:
We made corrections (grammatical and typographical) for errors detected when carefully re-reading our manuscript.