

Reviewer Report

Title: Female reproductive tract microbiota influence egg production in layer chickens

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Reviewer name: Ivan Rychlik

Reviewer Comments to Author:

This is a very good study to which I have only a few comments, mostly to microbiota part in which I am stronger than in chicken genetics. The only weaker part, but not weak, is the fact that the study was performed with only a single flock and was not repeated with different hens at all. I understand that it is impossible to repeat the study in another flock but for example you could have set up *Cl. leptum* PCR and check for its presence in reproductive tract in completely different birds.

Please consider the following points.

line 94, indeed laparotomy?

l.111 and 114, I do not understand what PCR kit you used. Please, reword.

l.253, 254, this sentence is somewhat compromised. Please check and reword.

l.342, rather verified, or confirmed, then detected. By the way, I am not sure whether you used data from the qPCR in the rest of the manuscript

l.388, these are not Cyanobacteria, this is chloroplast DNA from plants in the feed, you may check very recent paper Volf et al. Eggshell and Feed Microbiota Do Not Represent Major Sources of Gut Anaerobes for Chickens in Commercial Production. *Microorganisms* 2021, 9, 1480.

l.462,463, I would suggest alternative explanation in this case and this is living on expense of host mucus secreted polysaccharides, Sonnenburg, J. L. et al. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science* 307, 1955-1959 (2005)

l.559, check that *B. fragilis* might be a consequence of intensive human care, Kollarčíková et al. Different *Bacteroides* Species Colonise Human and Chicken Intestinal Tract. *Microorganisms* 2020, 8, 1483.

l.615, how can you know this? What if all of this the other way round, and I indeed believe that this is the other way, i.e. hens become of compromised performance, due to whatever factor, within but possibly also outside of those which you have monitored. This naturally results in decrease in egg lay but also in increased inflammatory response. Locally changed conditions due to inflammatory signaling change, infiltrating heterophils and macrophages produce antimicrobial peptides and reactive oxygen species and strict anaerobes will be the first bacterial species to decrease in a response of increasing oxygen concentration. What is cause and what is consequence. I do not know, I think that you do not know either, though you blame bacteria that these are responsible for the response.

l.631, similar to previous comment, I do not think that there is any downregulation. In high egg producers, there is basal, background expression of inflammatory marker genes. And these are induced in the hens with compromised performance. Be also careful, whether this induction since this could also be a cause of infiltration of macrophage with their specific expression profile, and you then mistakenly conclude on induction when purifying mRNA from a total complex tissue.

l.656, the same as above, be careful what is cause and what is consequence. Increase in reactive oxygen

species may affect the most strict anaerobes. When these are present, there is no inflammation. When these are eliminated by increase in oxygen concentration and all other inflammatory responses, this is explained that these bacteria are anti-inflammatory. These are not, these only dislike inflammation and oxygen.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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Are the conclusions adequately supported by the data shown? Choose an item.

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