Detail list of corrections/modifications undertaken in the MS in response to reviewers' comments

MS ID: Microorganisms-1065112

MS title: Gut microbial composition differs extensively among Indian native chicken breeds originated in different geographical locations and a commercial broiler line, but breed specific, as well as across breed core microbiomes are found

Reviewer's Comment	Authors' Response
REVIEWER#3	
In presented manuscript, the composition gut microbiome of 3 indigenous Indian chicken breeds (Nicobari, Ghagus, and Aseel) and commercial broiler hybrid VenCobb 400 were analyzed. As I reviewed few articles (microbiome analyses) with very poor bioinformatics and statistic treatments in last time, I must admire precise analysis in presented manuscript.	We would like to extend thanks for your valuable suggestions to improve the manuscript
However it looks like that authors flood down into the statistics without bringing the clear, simple, and easy to understand message through whole manuscript. Instead of simple clear results the same data are processed by various similar statistics and differences are lengthy described in results and differences among used statistics (e.g. Unifrac) are disputed in discussion Moreover many information are doubled – they are in text an also in figures.. Tables from supplement should be incorporated to main manuscript and then text can be simplified, shortened and pointed out From methodical point of view, I found 2 main	In compliance with the reviewers comment and also based on suggestions of the reviewer2 we have condensed the manuscript substantially and removed duplications of texts and Tables/figs Pl see the revised MS given in track change mode The disputed segment has been removed in the revised MS as suggested Pl see the revised MS given in track change mode One Table (richness and diversity) from supplement has been moved to main manuscript as suggested

faults inside this manuscript First is the use of chickens with different age. Microbiome is age related and difference between broiler (6 weeks) and indigenous breeds (35 weeks) is enormous. I am sure that his is a source of big portion of differences among them. Moreover, authors only partially discuss age as source of variability with statements about microbiome stabilization at 3 weeks. But this seems to be cherry picking as there are many studies about age/microbiome relation in birds.	The rate of growth and rate of passage of digesta are faster in case of broiler as compared to native birds. It is difficult to find an ideal equivalent age to compare fast growing broilers with slow growing native chickens. Studies on broiler gut microbiota beyond 6 weeks age are scarce and may not be of much interest to stakeholders as these birds are unlikely to be reared beyond 5-6 weeks. On the other hand at 5 or 6 week, native birds would have much lower BW and probably immature gut than broilers of same age. Our intent was to compare microbiome profile of these two groups of birds at marketable age (at end of their productive life) as this is essential to help designing strategies to modulate its composition not only to improve host health and performance, but also to control zoonotic agents that can contaminate poultry products. We agree with the reviewer's view that observed differences might also be partly attributed to age difference. We have added additional discussion and references to accommodate this point in the revised MS as suggested by the reviewer (L610-632)
Second problem is related to increased richness in Aseel gut microbiome. This increase may be related to high number sequences/ sample. No mater of clustering method or filtration higher sequence counts consistently brings increased richness in Illumina amplicon sequencing, thus richness may be artificially increased as Aseel samples had 5 time more sequences. I suggest that authors may try to decrease the number of input sequences in order to obtain comparable data.	In compliance with the suggestion of the reviewer we have reanalyzed the data after rarifying to minimum sample size and revised the Table and text accordingly(L218-220 and Table 2 of revised MS)
Authors showed the existence of breed specific complex microbial networks in all	We have reduced complexity of Fig 7 and S8 by increasing correlation threshold from

groups. However microbial network on figure S8 is hard to view and understand.	0.5 to 0.6 to focus on most important ones and to improve presentability. The revised figures appears OK now
While I doubt about the need of 4 NMDS analyses in figure 3 (one is sufficient);	We feel that this is OK and hence we would like to request the reviewer to give permission to retain all these figures
I recommend division of figure S8 into 4 smaller figures each for particular breed.	We have reduced complexity of S8 by increasing correlation threshold from 0.5 to 0.6 to focus on most important ones and to improve presentability. After the modification separate figures are not needed. Hope the reviewer will agree with us.
Figure 7 may be also divided, but it is readable in current form.	We have improved the presentability of the Figure as indicated above
Please consider also stacked bar charts for all samples at reasonable phylogenetic levels (phylum, class) instead or in addition to Krona	In compliance with the reviewers suggestion we have added a stacked barplot in Fig S1 in addition to Krona
Based on aforementioned facts major revision needs to be done in manuscript For publication of manuscript: Authors must explain why they used subjects of different age and thoroughly discuss it or remove the broiler from analysis. Authors must be more concise in text, mainly in results Authors should look over sequencing depth in relation to increased richness	We have taken care of all these suggestions as indicated above.