Reviewer 1:

Comments: The original manuscript under review entitled; 'Characterization of the gut microbial and metabolite alterations in the faeces of GK rats by a metagenome and untargeted metabolomics approach,' aimed evaluate the gut microbial and metabolite alterations in GK rat faeces based on metagenome and untargeted metabolomics. Although this manuscript is overall well structured and aims to better understand the gut microbiota and associated metagenome and metabolite structure of GK rats, the manuscript fails to show novelty/innovation in the context of the wider research field of T2DM and diabetes. In addition the clinical implications/applicability of results to humans are not adequately provided nor are the limitations relating translatability to humans. Lastly, background of the research to date regarding T2DM and the microbiota and associated metabolites in both humans and animals as well mechanistic insight regarding some of the results showing significant differences are not addressed appropriately. Firstly, the authors should sufficiently summarise and highlight key findings in the literature to date regarding T2DM and gut microbiota and metabolites in both animals and humans in the background, clearly showing the gaps in the literature and how this study aims to fill them and stating their hypothesis. In doing this, points of novelty of the study can then be provided – obviously the gut microbiota, metagenome and metabolite structure in GK rats has not been widely studied. Importantly the study needs to also demonstrate how these findings are applicable/translatable to humans and clinical implications (in the conclusion). Perhaps authors could touch on that in characterising the gut microbiota in GK rats and interpreting this against the wider findings in the literature to date, identification of certain species of microbiota and metabolites implicated in T2DM may help to lead to more personalised interventions and treatments that target the structure of the gut microbiota, metagenome and metabolites and how this then interacts with and influences aspects of metabolic health related to T2DM. Lastly, results such as the as the lower body weight seen in the final weeks in GK mice - (and HOMA-B) and the impact that these findings may have on the gut microbiota results should be discussed.

Response: The experts provided good guidance. We have added relevant contents to the Introduction and Results. We also further analysed the mechanism of action, which involves formation of a potential protease of the regulated metabolic pathways. Combined with the research results, we further explored the possible value of the clinical prevention and treatment of diabetes. We conducted an additional analysis of weight loss.

Reviewer 2:

Comments: 1. The introduction is too short. It would be helpful to supplement it with data on the importance of the gut microbiota to diabetes mellitus. 2. The study compared the gut microbiota in rats of different lines. However, the microbiota may have had differences even before the development of diabetes. It would be interesting to understand how the gut microbiota changes in GK rats as diabetes progresses. It might be possible to use 2 groups of GK rats in which the microbiota would be assessed at different times.

Response: The experts provided good guidance. We added content to the Introduction. Because rats should be in the same batch, the microbiota will be assessed at different times in future research.