RESPONSE LETTER

The authors would like to thank the reviewers for the comments made to our manuscript *'Towards a standard diet-induced and biopsy-confirmed mouse model of non-alcoholic steatohepatitis: Impact of dietary fat source'.* We have carefully addressed the criticisms point-by-point, our response is indicated below in italics. Changes in the manuscript are highlighted in yellow.

REVIEWER 1

General Comments:
In this manuscript, the authors reported a benefit of mice that fed a high-fat/fructose diet, in which trans-fat was substituted by palm oil, as a nonalcoholic fatty liver disease (NAFLD) model. In addition to the histological confirmation of fat deposit, active inflammation, and fiber accumulation in the liver, characteristic gene expression profiles and gut bacterial taxonomic shifts were presented in association with the disease development. The strategy is straightforward, and the results are clear. An unfocused presentation, however, diminishes the value of this study. The following are concerns that the authors may wish to consider.

Specific comments - Major concerns:
1. Although the authors presented the data of gene expression profiles and gut bacterial taxonomic shifts, there are not insightful interpretation nor discussion. If these data were included in this manuscript, the authors should thoroughly evaluate the results and provide the data in a comprehensive way.

Response: We have now expanded the Discussion on the liver transcriptome (p.25-27) and gut microbiome (p. 28-29) changes in GAN/AMLN ob/ob mice.

2. The authors describe in the introduction that a glucose intolerance is a hallmark of NAFLD. Although there is a clear difference in terms of a glucose intolerance between ob/ob mice that fed AMLN and GAN diets, histopathological findings of the liver are similar between these two groups. Please explain the reasons why the metabolic difference did not lead to either a phenotypic difference or gene expression profile in the liver?

Response: Only GAN ob/ob-NASH mice displayed impaired glucose tolerance. In contrast, the GAN and AMLN diets induced similar liver biochemical and histopathological changes in ob/ob mice. Also, liver transcriptome profiles, including reduced expression of various genes involved in lipid and glucose handling, were similar following GAN and AMLN diet feeding. We therefore speculate that extrahepatic insulin resistance may have contributed to impaired glucose homeostasis in GAN ob/ob-NASH mice. Accordingly, impaired glucose tolerance in ob/ob mice has been attributed to failure to suppress hepatic glucose production in conjunction with impaired glucose uptake in several insulin-responsive tissues, including muscle and fat, most likely precipitated by a defective triglyceride handling in these tissues. Although we in the present study did not specifically determine insulin sensitivity by hyperinsulinemic-euglycemic clamp techniques, this could suggest that the more marked adipogenic properties of the GAN diet could promote insulin resistance in several peripheral tissues to facilitate manifest glucose intolerance in GAN ob/ob-NASH mice. This is now considered in the Discussion (p. 25).

3. I believe that the aim of this study is to emphasize the benefit of a GAN diet in a NAFLD animal model comparing with an original AMLN diet. In this context, many aspects should be compared between two diets in the same genetical background. However, the authors performed several comparisons such as the clusters

of transcriptomes between ob/ob mice and C57 controls. If this article focuses on the effect of different diets to promote NAFLD, the control against a GAN diet should be an original AMLN diet in mice with the same background.

Response: The goal of the study was to compare to "normal" mice (C57Bl6 mice) rather than ob/ob mice on a different diet in order to maximize differences in biochemical, histopathological and liver transcriptome profiles. However, we agree that inclusion of control ob/ob mice on chow or regular diet would have been advantageous. Nevertheless, it should be noted that we have published sufficient papers on the phenotypic differences between AMLN and chow-fed ob/ob mice (Trevaskis et al., Am J Physiol Gastrointest Liver Physiol 302:G762-72, 2012; Kristiansen et al., World J Hepatol 8: 673-84, 2016; Tølbøl et al., World J Gastroenterol 24:179-194, 2018; Roth et al., World J Gastroenterol 24:195-210, 2018; Boland et al., World J Gastroenterol. 24:1748-1765, 2018) for comparison to the data presented in the present paper.

Specific comments - Major concerns:
1. Do not use an abbreviation such as AMLN from the beginning.

Response: The abbreviation AMLN refers to 'Amylin Liver NASH'. This is indicated at first appearance in the Introduction (p. 6), and now also written in full in the abstract.

REVIEWER 2

The authors try to design a new formula to establish animal model of non-alcoholic steatohepatitis in the manuscript. Authors analyze several critical the histopathologic characteristics by IHC and biochemical markers among of new model and referenced models. This model is very important in NASH studies. In this manuscript, the data could support authors conclusion, and the new model could be comparable to both models of ob/ob and trans-fat feeding. The manuscript organization is good.

No author response required.