Author’s response to reviews

Title: Neonatal diet impacts liver mitochondrial bioenergetics in piglets fed formula or human milk

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Reviewer reports:

(Reviewer 1): In general, the paper is well written, but some issues should be cover.
L=113 specify the number of the remaining animals.
A: The number of animals are included (n=30) in the text (line #116).

Figure 3: The authors didn't find any statistical significance. Please remove the legend *p<0.05
A: As requested we modified the sentence to “The data were analyzed by 2-tailed t-test to determine the significance between the diet groups.”

Please, specify in abstract and along the text that your data coming from male piglets
A: We have now included text about male piglets in the abstract and the methods (lines #30 and 97)

Sections should be carefully revised. In Introduction section the authors discussed the results, meanwhile in discussion section they are clearly introducing the paper... Also, the results should be more discussed (what explanation could be that in liver and ileum contradictory effect in Pparb with MH treatment?). Conclusions should be focused on the obtained data.

A: We appreciate the reviewer’s comment and have moved some of the intro to discussion and vice versa (lines #80-83, 278-282, 308-310). Overall, we feel that the current presentation adequately sets the stage and identifies knowledge gaps (Intro), while summarizing the results in comparison to the historic literature (Discussion). Second, we appreciate the reviewer highlighting tissue differences. We now include the following in the Discussion: “The current study design does not allow us to determine the specific mechanisms involved, but one speculation is that in the case of ileum there is more proximal exposure to diet- and microbe-derived tissue regulators, whereas metabolic regulation in the liver is an aggregate of gut-derived and systemic factors.” (lines #300-304)

A control group with natural milk from saws will help to decipher the role of milk. Human milk doesn't have the same nutrient composition than pigs, so they may produce an impact on mitochondrial expression... Unless, the authors should discuss why they don't use the "natural" milk

A: The sow fed piglets were always housed at the farm while both human milk and formula milk fed piglets are housed at the vivarium under the same environmental conditions. Inclusion of sow fed studies are often confounded by suckling compared with bottle feeding and by animal housing (e.g., farm, vivarium). These environmental and technical factors limit interpretations that can be derived from studying the effects of diet. We have demonstrated that microbiota composition of human milk fed piglet is similar to an infant fed with breastmilk (1. John J. Miklavcic, Thomas M. Badger, Anne K. Bowlin, Katelin S. Matazel, Mario A. Cleves, Tanya LeRoith, Manish K. Saraf, Sree V. Chintapalli, Brian D. Piccolo, Kartik Shankar and Laxmi Yeruva*. Human breast-milk feeding enhances the humoral and cell mediated immune response in neonatal piglets. J of Nutrition 2018, 148(11):1860-1870. 2. Lauren R. Brink, Katelin Matazel, Brian D. Piccolo, Anne K Bowlin, Sree V. Chintapalli, Kartik Shankar and Laxmi Yeruva. Neonatal diet impacts bioregional microbiota composition in piglets fed human breast milk or infant formula J Nutr. Dec 1;149(12):2236-2246). Microbiota have been shown to impact liver and muscle mitochondrial biogenesis and function. Due to limitations associated with data interpretation with sow-fed piglets, we chose to compare human milk and formula-fed piglets. We have added similar text to address the limitations of sow-fed model (lines #85-87).
Title should be modified. Meanwhile title says: "Neonatal diet IMPACTS liver mitochondria function..." the conclusions says "the data indicate that neonatal diet can significantly impact liver mitochondrial bioenergetics" so... it has to be according the results obtained...

A: We have modified the title to “Neonatal diet impacts liver mitochondrial bioenergetics in piglets fed formula or human milk” as per the recommendation.

(Reviewer 2): Dear Authors,

The article points to a subject of important discussion. However, I think that following ethical issues in consideration of breastfeeding guarantee policies, in the face of indisputable supremacy, due to numerous factors already pointed out in the literature, this point should be raised in the introduction. I believe it is fundamental, even though it is an experimental study.

A: Text in the introduction has been modified to “Breastfeeding has been shown to have positive impact on the body’s physiological systems, including the immune system and metabolically-important tissues such as liver, adipose, and cognitive centers in the brain [1, 2]” (lines #52-54)

Due to the type of data analysis (basically descriptive), I think that more robust statistical analysis, even if it is an experimental study, would add to the study. I believe that the conclusion does not clearly answer the objective.

A: We have conducted appropriate statistical analysis for this experiment, since comparisons were between two groups (2-tailed t-test). Our null hypothesis is that the mean of HM is not different than MF group for the outcomes measured. Thus, a two-tailed test was used. We have added the text “Our data demonstrate that neonatal diet impacts liver mitochondrial bioenergetics phenotypes. In addition, in the presence of ADP, mitochondrial respiration was increased in MF piglets relative to HM-fed piglets, suggesting that formula feeding led to a higher innate hepatic ATP turnover (lines #308-311).