Open Review (x) I would not like to sign my review report
( ) I would like to sign my review report

English language and style ( ) Extensive editing of English language and style required
( ) Moderate English changes required
(x) English language and style are fine/minor spell check required
( ) I don't feel qualified to judge about the English language and style

Yes Can be improved Must be improved Not applicable

Does the introduction provide sufficient background and include all relevant references? ( ) (x) ( ) ( )

Is the research design appropriate? ( ) (x) ( ) ( )

Are the methods adequately described? ( ) (x) ( ) ( )

Are the results clearly presented? ( ) (x) ( ) ( )

Are the conclusions supported by the results? ( ) (x) ( ) ( )

Comments and Suggestions for Authors

In the manuscript 'Mammary Gland Transcriptome and Proteome Modifications by Nutrient Restriction in Early Lactation Holstein Cows Challenged with Intramammary Lipopolysaccharide’ the authors aimed to test the hypothesis whether ‘undernutrition in early lactating cows would modify the inflammatory response at mRNA and protein levels’. It is not clear what is the practical meaning of this study. The aim of the study seems also to be not fully supported by the observation that undernutrition generally modifies the inflammatory response in the early lactation since, as the authors mention, this is also hypothetical (line 50: ‘...are likely to influence immune function’, line 56: ‘...that might influence immune system function’). Overall I have the impression that these are the secondary results of another study. The manuscript requires revision in some aspects as depicted below.

Response: Thank to the reviewer for the commentary. It was observed that cows in the early lactation state are more susceptible to inflammation. The literature reported that NEB which occurs in that moment can play a role in this susceptibility. However, because of the complexity of the phenomena, NEB role in inflammation is still unclear. Our aim was to
provide more new information which could help to understand this process. These knowledges are important to help to prevent and fight against MG inflammation especially when nowadays it is big pressure to diminish the use of antibiotics in farm animals. The better understanding of this process can help to breed animals and make genetic selection forward more resistant against inflammation. We used hypothetical statements because gene expression detected as modified still poorly documented. The hypothesis in this work was based on the literature and we focused on the role of DEG in inflammation. Indeed, our knowledge of function of all gene is still incomplete and sometimes there few information about them but its role can be important just still not fully understood.

1. Study design – why qPCR was conducted before microarray analysis. Usually it is the other way round – qPCR is conducted to verify most significant changes observed in microarray study.

Response: We clarified this point at the end of the introduction to explain our approaches, the title of the 2.2 section, and in the 3.1 section.

2. Study design – there is some inconsistency between the aim of the study, the hypothesis and the methodology. The aim was focused specifically on the immune function but the „omics“ analysis yielded data on many different processes. In fact, the process which was mostly affected by undernutrition at the transcriptome level, was associated with metabolism and the authors paid much attention to describe changes in metabolic processes induced by undernutrition after LPS challenge. Similarly, at the proteome level, the mainly affected process was not associated with immune response, but with protein synthesis and much of the discussion referes to this observation. From this point of view, the main aim of this study should be to investigate which processes are generally affected by undernutrition after LPS challenge.

Response: We agree with the reviewer. We thought to have already clarified this point (with the last sentence of the introduction of the previous revision). However it appears to be not enough clear. So we added a sentence at the end of the introduction to clarify this point.

3. As already mentioned, what is practical meaning of this study? How may the industry benefit from this study results?
Response: This study was performed with the aim to increase our knowledge on the effects of NEB occurring in early lactation which is the time of high risk of mastitis. This knowledge will be helpful for genetics studies. We added this point at the end of our manuscript.

Minor comments

1. Figures 1 and 2 – percent values would be helpful.

Response: We added them.