

Jindřich Chmelař

Oponent review of the master thesis of Bc. Matěje Kučera named

Influence of dietary components and redox enzymes on intestinal microbiota proliferation in the tick *Ixodes ricinus*

The work has standard extent and division into 6 sections – Introduction, Aims, Material and Methods, Results, Discussion and Conclusion. There is also supplemental material, which is not part of the topic, but deals with important methodological work on the optimization of artificial tick feeding. The work has 47 pages plus 5 pages of supplement.

The work is written in acceptable English for a candidate on Master's degree. Despite a number of grammar or syntax mistakes, the work reads well and the author will have for sure the opportunity to improve his writing in the process of publishing a scientific paper.

Introduction:

In the introduction, the author describes sequentially tick digestion of hemoglobin, importance of microbiota and various mechanisms of tick/arthropod immune system, finishing by the description of two enzymes involved in ROS production and antibacterial control in arthropods. Obviously, very little is known about these two enzymes in ticks, however, is there more knowledge on the role of oxidative stress and ROS in tick antimicrobial defense or its physiology?

Aims of work:

Aims are well defined, but maybe too broad. There are actually three topics – impact of hemoglobin, impact of dietary composition and characterization of DUOX enzyme and each topic could actually cover full thesis, if approached more thoroughly.

Methods and material:

All used methods are described in details, nevertheless, some important detail are missing. I have several points to this part.

1. I miss the citation of the formulation for membrane mixture as I did not find it in Kröber and Guerin 2007 reference.
2. How the manual blood defibrination is done?
3. The glucose concentration (and overall diet formulation) was assessed by optimization or from the literature? – This is actually general comment to methods part: Some methods used in the work lack proper reference (e.g. why flagelin was used as housekeeping, how primers and probes were designed, reference to relative qPCR evaluation method)
4. Tables with ligation and restriction miss actual concentrations or at least reference of specific kit and enzyme producer, so that used concentration could be found.

Results:

Several different results are shown, from which I find very interesting the reciprocity of bacterial and borrelia load in tick gut and also the effect of Ir-DUOX silencing on tick feeding and survival. To this part I have following questions?

1. Borrelia clustering after treatment with complement inactivated serum is also present. The “rosetta” formation in figure 3C is something typical? And what it implies, if something?

2. How do you recognize symbiotic tick gut microflora from infection?
3. Reciprocity in borrelia/bacteria ratio in tick midgut is interesting result. What experiment would you design to show causal relationship?
4. Samples for determining RNAi efficiency were pooled together in Figure 7. Why individual samples were not used instead? There is lack of information from where the expression leakage in figure B and D origins.
5. Again in Figure 8, there is pooling of guts. Why? I think it leads just to loss of statistical power.

Discussion:

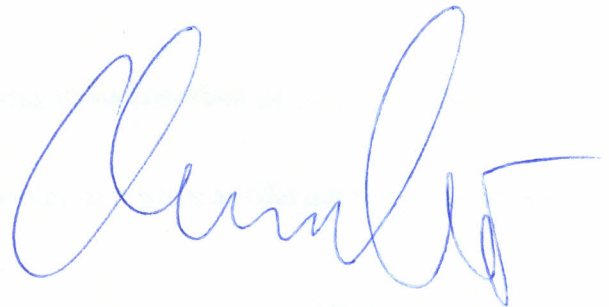
Discussion is well written and the results are nicely commented in the light of the literature. Complement is important defensive mechanism, however in addition to complement and hemoglobin, there is another factor that is not taken into consideration in the work – cells. Is it possible that the difference in affecting bacterial load in ticks between blood and serum is caused by the cell content or even by the activity of immune cells (if they are still alive in the artificial blood meal)? To prove hemoglobin role, I would use serum with pure hemoglobin without cellular fraction.

Author writes that borrelia may need heme for its proliferation. What is heme needed for in bacteria, what could be its role in borrelia?

Overall I consider the master's thesis of Matěj Kučera as interesting and useful for further research. Especially the observation that borrelia and gut bacteria numbers are negatively correlated and the effect of Ir-DUOX on tick feeding deserve further attention. The work raised several important questions and it is pity that they were not addressed.

I recommend the thesis for defending and suggest the grade 1- or 2, depending of author's performance at the defense.

In České Budějovice, 11.5.2015

A handwritten signature in blue ink, appearing to be 'A. Kučera', written in a cursive style.

Evaluation of Master thesis:

Influence of dietary components and redox enzymes on intestinal microbiota proliferation in the tick *Ixodes ricinus*

by Matěj Kučera

Referee: Eva Horáková

In this Master thesis, Matěj has carried out studies on intestinal microbiota of tick *Ixodes ricinus*, focusing on spirochete *Borellia afzelii* during artificial feeding with different diets. In addition, he RNAi knock-down dual oxidase and studied its phenotypic outcome in *Ixodes ricinus*. Last but not least he optimized the artificial feeding of nymphs which may positively influence the elaborate experiments on studied organism.

The thesis has regular arrangement and the individual chapters such as Introduction, Results etc. are well balanced. On the other hand the work it is a bit fragmental, but I think that the authors sealed individual projects quite nicely. The work is written in English and I find it quite OK. The writing has minimum typos, sometimes sounds a bit clumsy and needs some polishing.

Most of the experiments are still sketchy and need to be followed in order to bring more answers than questions. I am aware of the fact that the time of the Master student is very limited and especially the one working with ticks. Yet, it would be nice to see the future perspective of the individual topics during the defence presentation.

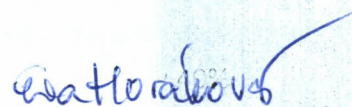
General questions to the student:

1. Overall, I am missing the general view of the topic studied, the schematic representation showing the crosstalk between, heme, ROS (Duox) and microbiota in the tick gut possibly compared to other insect studied in the literature. If you can provide such scheme it will be very helpful.
2. In the discussion section 5.3. you wrote: "Previously we determined, that *I. ricinus* code for a good ortholog of predicted *I. scapularis* genome. The sentence doesn't make sense to me, I suppose you ment the IrDUOX ortholog. Anyway how would you define a "good ortholog"?
3. I am missing the errors bars in your qPCR experiment (page 30). How many times was the experiment done?

4. In your work you are questioning the specificity of the *ir-duox* antibody. Unfortunately the data are not shown. Can you show the western based on which you made this conclusion? How was the antibody prepared?
5. Throughout the thesis you are mentioning ROS quite frequently. Did you try to measure the ROS production in your study on ticks?
6. Are there other proteins eliminating reactive oxygen species present in *I. ricinus*? Is it known how they influence the microbiota in guts?
7. How come peritrophic matrix is needed in the tick gut, if all the digestion is happening intracellularly?
8. Do you think that the data on artificial feeding of nymphs where you show 40% efficiency is good enough for publication? How far do you want optimize your method? How efficient is the feeding of adults? Where you able to infect the nymphs with *Borellia* by the given method?

To my opinion this thesis meets the criteria for a Master degree and I would like to grade the thesis based on Matej's defence.

20. května 2015 v Českých Budějovicích


Eva Horáková