Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Katharina Lehr

Thesis title: Trehalose as an important energy source for immune cells in *Drosophila melanogaster*

Supervisor: doc. Mgr. Tomáš Doležal, Ph.D.

Referee: Mgr. Josef Houser, Ph.D.

Referee`s affiliation: Central European Institute of Technology, Masaryk University, Brno

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	1
Graphic layout of the text and of the figures/tables	0-3	1
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	1
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
Formal requirements – points in total		11
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	0
Logic in the course of the experimental work	0-3	3

* Choose one

1 Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		17
POINTS IN TOTAL (MAX/AWARDED)	48	28

Comments of the reviewer on the student and the thesis:

The bachelor thesis by Katharina Lehr deals with an interesting topic of trehalose utilization in insect body and its importance for immune system. The topic is highly important as the insect immune system can also serve as a model for human-oriented research.

The thesis contains several issues that need to be mentioned. The language and grammar used is not always clear resulting in misguiding the reader. There appear typos and missing words, more care should have been paid also to proper abbreviation usage. The whole thesis generally looks disordered, sometimes even chaotic. Chapter 1.1 Introduction contains partially Abstract and is highly heterogeneous. Chapters 3.4.3 – 3.4.5 shall be subchapters to 3.4.2 and not on the same level. In several parts of thesis, it is not clear, whether some external information is used (than there is citation missing) or whether it is a result of experiments (but written in Introduction part). Figures and tables are not always numbered according to their appearance in text, the list of abbreviations is not alphabetically ordered, as well as multiple references in given place are not always arranged according to numbers. More attention could be paid to graphical layout, especially in figure arrangement (e.g. "merged" Fig. 1 and 2). Name of the figures (graphs) should be part of caption and not as individual line above. Figure 15 (and corresponding results) should precede Figure 14. Also a part of results considering the initial stages of work are missing even though they are commented in the text. Tables in attachment are not well described and there is no reference to them in the text.

The experimental methods are mostly satisfactory described, however there is frequently missing source of used material (organisms, media, primers) or composition (media, buffers), no mention of the type of used microscope or spectrophotometer. Formulations such as "were provided by a student from the lab" without further specification should never appear in scientific text. Few points are not clear or confusing (DNA was diluted after qPCR, DNA was mixed with master mix that already contained DNA, etc.). Abbreviation hpi (hours post-infection) is counterintuitive when used for non-infected control.

Mainly relevant and up to date sources are cited, however the list of references is non-uniform, both abbreviations and full journal names are used, some author names are used only as initials and student should avoid using German in English text.

Main formal (and also practical) problem of the thesis is the absence of discussion. Even though it is a mandatory part, there is no such chapter (neither solo or connected to results). Individual sentences that could be considered as discussion are scattered throughout the thesis including the Introduction and Conclusion parts. Very few observations are discussed in result part with only one scientific paper mentioned. Some conclusions made in the thesis are exaggerated or

not well supported by experiments. Here, the missing discussion could have support the conclusions or explain them in further details.

The thesis in overall meets the minimal criteria for bachelor thesis considering the theoretical part, methodology, aim of the study and received results. Unfortunately, I cannot recommend it for defense due to missing discussion part that is obligatory based on official rules for thesis at the Institute of Chemistry, Faculty of Science, University of South Bohemia.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1) In page 3, the *Leptopilina boulardi* eggs are said to be able to form "a virus-like particle". This does not match the common sense of "virus-like particle" as being used in virology. Can student explain the statement?
2) How were the used primers designed? What were the considered criteria and which (if any) software tool was applied?
3) Why was the sample after PCR kept at 12 deg instead of more frequently used 4 deg?
4) During the PCR optimization with primers FW1+RV1 (Figure 13), a clear difference between two tested samples can be seen. What could be the cause of such result and the consequences for the choice of "optimal" PCR strategy?
5) Obtained PCR product for negative control (Figure 14) corresponds by its size nicely with the predicted product based on Table 9. Does it mean that the "negative" control is the only positive sample or is there some mistake in results?
6) Based on qPCR results, the increased trehalose uptake and processing is commented as "preferred" by immune cells. However, no comparison is done with glucose utilization under the same conditions. Can student comment on this issue and possibly compare the observed data with so-far published works on glucose, if they exist?

Conclusion:

In conclusion, I

~~**recommend**~~ **/ do not recommend***

the thesis for the defense and I suggest the grade **4 .**

In Brno, 10th September 2019

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signature

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OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: **Katharina Lehr**

Thesis title: Trehalose as an important energy source for immune cells in *Drosophila melanogaster*

Supervisor: **doc. Mgr. Tomas Dolezal, Ph.D.**

Referee: RNDr. Alena Krejci, Ph.D.

Referee`s affiliation: **University of South Bohemia, Faculty of Science**

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
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Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		16
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		21
POINTS IN TOTAL (MAX/AWARDED)	37	(0-48)

Comments of the reviewer on the student and the thesis:

In her thesis, Katarina Lehr investigates the intriguing connection between trehalose and its usage by immune cells during the infection of *Drosophila* which is an unexplored topic in the field and a new concept emerging from the lab of Tomas Dolezal. Catharina used a whole range of molecular and cellular biology techniques and generated a solid set of data. It is true that from an outsider view the submitted thesis seemed to be incomplete because there was not a single line of Discussion. However, from reading the thesis it became obvious that the results and discussion were connected in the same section, unfortunately entitled just Results instead of Results and Discussion. It still is strange that only one work is discussed and compared to Katharina's new results but when I searched Pubmed myself it turned out that there are not many other relevant publications where the role of trehalose during immune response would be explored. It is questionable whether the discussion should have been broader, not concentrating on trehalose alone but perhaps on the immune system energetics from a wider perspective. For an experienced scientist this would not be a problem but for a first scientific attempt of a student this was probably confusing. It certainly is unfortunate to submit thesis where only one paper would be cited and compared in the Discussion section. There are 32 citations in her thesis so Katarina must have read relevant literature but she was probably scared not to be focused and discuss her results too broadly. Katarina was most probably desperate and did not know how to face the lack of the related literature, maybe also with the stress of the ticking submission deadline for the thesis. I hope and believe that she took a lesson from it and not only she will be more careful next time but she will also not leave things for the last minute.

The thesis is otherwise nicely written. The introduction is brief but focused, although it contains some inaccuracies ('Trehalose is used by immune cell to produce energy; the other cells are only able to utilize glucose') and it is difficult to decipher important information about gene names (Tret and Glut). The Aims are well defined and fulfilled in the experimental part. The description of the methods is sometimes lacking details (how many larvae were infected by the wasps in each experiment?) or uses nonstandard ways of expression (like writing down crosses with stock numbers instead of the actual genotypes). It wood have been useful to read the chapter once more before submission in order not to mix mL and uL (p.12). The results are well described and quite nicely discussed in the context of Bajgar's work. It is obvious that more biological replicates are needed to solidify the data but I understand the lab time of the Linz students is limited and Katarina chose to try different techniques rather than replicate the same experiments. Overall, the work generated useful data although it is quite brief and extra day or two of editing could improve the impression. Nevertheless, I consider it sufficient for the defence of a bachelor degree.

Katarina generated very nice and useful set of results and by my opinion the lack of some of the formal requirements of the thesis should not prevent her to pass the defence. I would be more willing to suggest failing of a student who wrote a page of some sort of weak discussion but did not produce much data or if it was obvious that the student does not really understand the background or purpose of her project. This is not Katarina's case. There are plenty of smaller or bigger inaccuracies in her thesis but in comparison to other students and presuming a good impression from her actual defence, I would suggest the grade very good.

Some questions that can be discussed during the defence:

Technical questions:

- why did you use the extension time of 1'30" for the real time PCR if your PCR products were only 100-200bp long? Why did not you use melting analysis at the end of the reaction? How do you decide about the length of the extension time to use and what is the melting curve good for?
- Why did you use 6 ul of 2x SYBR green Master mix reaction of a total volume of 10ul?
- you say on p.17 that 'As a control, the fly strain 1498 was also infected and lamellocytes were counted'. Are you sure it was like that? Or did you cross this strain to any flies?
- nowhere in the Method section it is described how exactly you calculated the expression levels from the Ct values. Can you explain?

Questions for discussion:

- Could you speculate why there is a peak of increased glucose levels at the first few hours after infection, as observed f.e. by Bajgar et al. if according to your model trehalose is the main source of sugar for activated immune cells? Could glucose be used at the beginning of infection and trehalose in later phases of the infection? Please correlate your trehalases expression data to the expression of trehalose transporters and glucose transporters, if the data are available.
- Is trehalose used in other organisms apart of Drosophila? Would you expect to play similar role during the immune response? And how about organisms that do not contain trehalose in circulation?
- Could trehalose by used for other purposes than an energy source?

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade very good **depending on the performance during the defense.**

In **Ceske Budejovice** date **5th September 2019**