



Přírodovědecká
fakulta
Faculty
of Science

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

OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Bc. Simona Fišerová, BSc.

Thesis title: The effect of *Ixodes ricinus* tick serpin on the cytotoxic function of natural killer cells

Supervisor: Mgr. Jaroslava Lieskovská, Ph.D.

Referee: RNDr. Martin Palus, Ph.D.

Referee's affiliation: Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic; Veterinary Research Institute, Brno, Czech Republic

	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
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
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	2
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	3
Logic in the course of the experimental work	0-3	3

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	2
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		20

POINTS IN TOTAL (MAX/AWARDED)	48	(36)
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Comments of the reviewer on the student and the thesis:

The thesis of Simona Fišerová, titled "The effect of *Ixodes ricinus* tick serpin on the cytotoxic function of natural killer cells" fulfills the requirements of the diploma thesis quality (reflected in the point assessment above). The main aim of the thesis was optimization of method for the measurement of the cytotoxic activity of prepared murine spleen cells. The author took into account several parameters which could affect the measurement. However, obtained data do not support the initial hypotheses and raise additional questions for further experiments.

Suggestions and questions, to which the student has to answer during the defense.

1. Section 4.5 the author has written: "...one-way ANOVA with unpaired t.test (chapter 5.4), Tukey's Multiple Comparison test (chapter 5.5) and Tukey's Post-hoc test (chapter 5.6)." I would like to ask the author to explain the difference between Tukey's Multiple Comparison test and Tukey's Post-hoc test.
2. I would suggest to avoid word combination; "isolation of effector cells" since these were not really isolated but rather "prepared." It is clear from the text that the author is aware that she was testing cytotoxic activity of spleen cells and not just natural killer cells. Therefore, my question is:
What are the cell type population ratios if the spleen cells are prepared with and without Poly I:C treatment, as written in 4.1.2? What would be the method of choice to get isolated natural killer cells suitable for this cytotoxic activity measurement (include reference)?
3. In the discussion, the author compares method used for cytotoxic activity measurement, calcein-AM release method used in this thesis with ⁵¹chromium release method used in bachelor thesis Beránková 2015. The difference in the effectiveness level of measured cytotoxicity of NK cells was 24 % for calcein-AM method vs. 62 % for ⁵¹chromium release method. Based on this comparison the author stated: "As conditions of experiments were otherwise similar it seems that calcein-AM release method is only suboptimal." However, the effector and target cells ratio was different. In the author thesis 50:1 (24%) and in the Beránková thesis 100:1 (62%). Is it possible that this ratio could affect the effectiveness of used methods? Were different ratios tested for calcein-AM method?
4. The author presumed that IL-2 activates mainly NK cells (spleen cells) and the production of IFN-γ is a feature of activated NK cells. However, the experiment did not result in a

noticeable activation/stimulation of NK cells. What are possible explanations why IL-2 has no effect on activation of treated cells?

5. The presented data show an increase in IFN- γ production which occurred in the presence of IRS-4 and also the increase of NK cells (spleen cells) cytotoxic activity for IRS-4 treatment. The author suggests as possible cause of the activity increase the synergistic cumulative effect of IRS-4 and LPS. Please, suggest an experiment that would help to confirm/reject this hypothesis.

Mistakes, which the students should avoid in the future:

It would be beneficial for the thesis if more time has been spent with additional corrections concerning the formal part of the thesis:

Capital letters used for chapter 3.1 and 3.2; too small size of Figure 1; avoid comment such as "Such a brief description is for the purpose of my thesis efficient...;" *Ixodes ricinus*" instead of "*Ixodes Ricinus*"; "Sheep Thick" instead of "sheep tick"; "specific-pathogen-free" instead of "pathogen-free"; some Latin words are not in italics (Ixodidae; et al.;...); centrifugation forces should be in g-force unit rather than RPM; provider of assay possibly cat. No or version of used software should be listed (e.g. section 4.5; 4.3;...); author names in references are not in uniform style (reference 42 is without year)...

More attention should be given to use and explanation of abbreviations: some are not explained in the text e.g. TBEV and TBE and are used incorrectly since TBEV is not illness (same for RFU, RFV,...); avoid using just abbreviations in headlines (content and sections); some abbreviations are explained and used unnecessarily (BOFES, ATB, GL ,...); avoid explanation of abbreviations by new abbreviation as in section 4.1.3,...

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade ²

In České Budějovice date 16.7.2020

signature

² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).