



Přírodovědecká
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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: Manuel Stammeler

Thesis title: α -GAL immunity in fish and its potential for modulating the effect of parasitic infections

Supervisor: Mgr. Astrid Holzer, Ph. D.

Referee: RNDr. Jarmila Štěřbová

Referee's affiliation: Department of Chemistry, Faculty of Sciences, USB & Institute of Parasitology, Biology Centre of CAS

	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	1
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	3
Language and stylistics, complying with the valid terminology	0-3	3
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		15
(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	3

* Choose one

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

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	3
Practical requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	48	39

Comments of the reviewer on the student and the thesis:

The bachelor thesis submitted by the student Manuel Stammmler aimed to study α -GAL immunity in fish and its potential for modulating the effect of parasitic infections. The thesis is focused on the stimulation of protective immunity of common carp *Cyprinus carpio* against *Sphaerospora molnari* through the administration of probiotic treatment containing α -GAL-rich *Escherichia coli* strain. The topic is actual as *S. molnari* causes systemic immune responses and haemolytic anaemia in common carp and the study of α -GAL immune response is a promising field of interest for many researchers.

The thesis has 32 pages and it is divided into an Abstract, Table of contents, Introduction, Aims of the study, Methods, Results, Discussion, Conclusion, and References. The extent of the thesis is satisfactory, and the text is coherent and mostly clearly written. Despite very good writing skills, several mistakes are unfortunately present like missing gaps or full stops. Despite these minor mistakes, the formal side of the thesis is on a very good level.

As far as the individual parts: the Abstract is well written with appropriate length.

The Introduction consists of only two and a half pages and in my opinion, is the weakest part of the thesis. The text is written understandably, and the reader gets the information in the chapters about Myxozoa, including *S. molnari*, the immune system of Teleostei, and the α -GAL epitope. As the introduction is meant to give information needed to understand the rest of the thesis, I am missing information about the common carp, which is the organism of interest or about α -GAL-rich *E. coli* strain that is used for the stimulation of carp's immunity.

The aims of the study are clearly stated, filled in three points.

The methods are clearly described, and the text is comprehensible so that the reader can repeat the individual experiments. Some of the abbreviations are not explained, for example SPF carp, FACS, DEAE cellulose. I also miss information about the used chemicals – in the subchapter Flow Cytometry, the manufacturer of RPMI medium, primary and secondary antibody, and DiOC6 is absent. In the subchapter ELISA, a final concentration of chemicals instead of their weights should be stated for the bicarbonate-carbonate coating buffer. Again, no manufacturer is listed for the antibodies and commercial buffers/solutions (1-Step™ Ultra TMB -ELISA Substrate Solution). On the other hand, the subchapter Treatment groups and experimental timing is very well written and comprehensive.

The results section deals mainly with the measurement of changes in the composition of individual blood cell types using flow cytometry, determination of antibody titres using ELISA, and quantification of *S. molnari* using qPCR, which together give a comprehensive picture.

As the fish and the types of treatment were divided into six different groups and the samples were collected in different time intervals, it is sometimes difficult to orient through the results. The groups are marked with letters as well as with the names, but some graphs are described only by names (Figs. 9, 10, 12, 14) and some by letters (Fig. 20), which makes it harder to understand the results. For the reasons mentioned above, it would be better to describe the results from Flow cytometry more in the text below the individual graphs. I have also several questions that are in the next section.

The discussion has two and a half pages with twelve citations which is adequate. The student goes through his results fluently, discusses them with other published results and gives the reader a possible explanation of his results.

The Conclusion section is well and clearly written.

References refer to relevant articles and sources and older and newer articles are equally present, nevertheless, they are not uniform. For example, in some cases, the name of the journal is missing (Lom et al., 1983; Pacheco et al., 2021). For future work, I recommend using some reference manager software like EndNote or the freely available Mendeley.

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

Q.1 In the Introduction, there is no information about common carp, the subject of your study. Could you present some basic information about *Cyprinus carpio* and why is it important to protect it before parasites?

Q.2 Chapter 4.2. Flow cytometry – Why is it important to exclude doublet cells from the analysis?

Q.3 ELISA Fig. 18: Could you explain, why is the titer of antibodies in negative control (and other groups) so high at dpi -36 (pre-bacterial treatment)? Does it affect your conclusions? Did you use technical triplicates of the samples for statistical validation of the results?

Q.4 In the Introduction you write that *S. molnari* infects skin and gills. Why did you use liver samples for qPCR?

Conclusion:

In conclusion, I

r e c o m m e n d / ~~d o n o t r e c o m m e n d~~*

the thesis for the defense and I suggest the grade 2 .²

In Ceske Budejovice date 12. 9. 23

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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).