



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Zuzana Bláhová, Dipl.-Ing.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Martin Pšenička, Ph.D.
Title of PhD thesis: Regulation of biosynthesis of LC-PUFA in fish	

REVIEWER:

Surname: Makhutova	Institution: Siberian Federal University, Krasnoyarsk, Russia
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Please describe your professional relationship to the PhD student: none	Please describe your field of expertise: aquatic ecology, lipidology, fatty acid metabolism in fish, trophic relationships in aquatic ecosystems

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

The importance of the work is beyond doubt since it concerns the topic of omega-3 PUFA deficiency in human nutrition. Two omega-3 PUFAs, namely eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acid (DHA), have a high physiological value and play a key role in human health. Health international organizations (e.g., World Health Organization) recommend the consumption of 0.5-1 g of EPA + DHA per day for the prevention of cardiovascular and other diseases and disorders. The main food source of EPA and DHA for humans are aquatic ecosystems and, primarily, fish. The humanity is growing at a high rate. Already, the commercial catch of fish and seafood has reached its limits. At the same time, according to the Food and Agriculture Organization, a person on average consumes 10 times less than the existing recommended norm. It is obvious that already now humanity is experiencing an acute shortage of these physiologically valuable substances. There are several ways to reduce the deficiency of these important substances, for example, aquaculture of those fish species that have an increased level of their own PUFA synthesis or the production of transgenic organisms that efficiently synthesize PUFAs. Leading scientists in this field, such as Douglas Tocher, Oscar Monroig, Johnathan Napier and others, are working on a solution to this problem. Thus, PhD thesis of Zuzana Bláhová, aimed at studying the genetic and biochemical basis of PUFA synthesis in fish and revealing the effect of genetic modification of fish on their reproductive capacity, is of fundamental and practical importance. The experiments on partial knockout of *fads2* gene in *Danio rerio* and subsequent tracking of the reproductive activity of genetically modified fish were carried out for the first time, so the novelty of the work is beyond doubt.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent $\frac{1}{4}$ – $\frac{1}{2}$ page):

The dissertation consists of 4 chapters. The first chapter provides general information about fatty acids and their synthesis and formulates the aim and objectives of the work. The second chapter presents the data of a published review paper, which summarizes the current data on the synthesis of fatty acids in fish with special attention to the key enzyme of long-chain polyunsaturated fatty acid biosynthesis - FADS2. The third chapter presents the data of a published research paper reporting an experiment on the partial *fads2* gene knockout in the *Danio rerio* (model fish) and the effect of this gene modification on reproductive system of the fish. The fourth chapter contains a short discussion of the results obtained and prospects for the future, a conclusion, an abstract and information about the author and supervision plan. The first section is poorly structured, contains many small mistakes and conflicting data. However, these drawbacks only slightly worsen the impression of the dissertation since chapters two and three are done at a high level - the literature is carefully reviewed, the experiment is well thought out and carried out, logical conclusions are drawn. The fourth chapter perfectly complements the work and forms a holistic view of the dissertation. Both papers have been published in the high-quality Q2 journals (Web of Science). Publications are fully consistent with the topic of the dissertation. The assigned objectives have been completed.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

Specific remarks referring to Chapter 1.

Section 1.2. - In the first sentence, the author describes fatty acids in general, but gives the formula for saturated fatty acids only rather than the general formula. When describing the structure of the FAs with the example of the EPA, some words in the sentence are omitted and there are superfluous ones (it is an example of bad proofreading of the text).

Section 1.3. The synthesis of fatty acids is not fully described. For example, the information on eukaryotic pathway for the synthesis of C18 FAs in plants has been omitted; there is no mention of the main PUFA synthesis pathway in bacteria - the polyketide pathway.

The author provides conflicting information. "In contrast, no living animal has been shown to have enzymes which could desaturate fatty acid chains beyond the 9-10 position of the poly carbon fatty acyl chain" and "Recently, multiple invertebrates have been confirmed to produce PUFAs de novo and further biosynthesize them into LC-PUFAs similarly to single-cell microorganisms".

Section 1.5. This section describes the *fads2* gene and the desaturase FADS2 it encodes. However, if in previous sections fatty acids were described in detail, starting with the nomenclature, here there is no complete information about the enzyme and its functions. Yet, the main focus of the dissertation is on this enzyme. The author should have dwelt on FADS2 in more detail.

Some of the ecological judgments given in this chapter contain few or no references to papers, although there are many such papers.

The figures in this chapter deserve special attention. This chapter contains 6 figures that are not referenced in the text. Although in one place there is a reference to figure 3, but it is clearly erroneous. The figures themselves have minor mistakes. For example, the first double bond is not labeled in Figure 3; in Figure 4, there is no



identification of the position of double bonds in the formulas; Figure 5 does not reflect all PUFA synthesis pathways; two figures have the same number (Fig. 5).

On the whole, Chapter 1 gives the impression of being badly edited and proofread by the author.

There are no comments for other chapters.

FINAL RECOMMENDATION

- ☒ PhD thesis can be recommended for defence
- ☐ PhD thesis can be recommended for defence with reservations
- ☐ PhD thesis cannot be recommended for defence

04.04.22, Krasnoyarsk

Date and place

Olesia Markytova Slof

Name and signature