OPINION

by prof. Albena Georgieva Jordanova, PhD, Faculty of Medicine,

Sofia University "St. Kliment Ohridski"

of a dissertation for awarding the educational and scientific **PhD degree**

field of higher education: **4. "Natural sciences, mathematics and informatics"**
professional direction: **4.3. "Biological Sciences"**
PhD program: **"Medical Biochemistry"**

Author: Assistant Professor Maria Atanasova Choneva

Form of PhD study: independent preparation

Department: Medical Biochemistry, Faculty of Pharmacy, Medical University - Plovdiv

Topic: Effects of prebiotic oligosaccharides and aerobic training on metabolic and behavioral disorders in an experimental model of type 1 diabetes

Research supervisor: Assoc. Prof. Anelia Veselinova Bivolarska, PhD, Medical Biochemistry, Faculty of Pharmacy, Medical University - Plovdiv

1. General presentation of the procedure and the PhD student

The presented set of materials is in full compliance with article 70 (1) of Section I. Acquisition of educational and scientific PhD degree and scientific degree "doctor of sciences" at Medical University - Plovdiv; Regulations of Medical University - Plovdiv dated 28.01.2021 and includes the following documents:

- application to the Rector of MU-Plovdiv for startup of the procedure for the defense of a dissertation work;
- curriculum vitae in European format;
- higher education diploma and its application;
- orders for enrollment in PhD studies (25.07.2023) and for deduction with the right of defense (01.04.2024);
- protocol for exam for PhD minimum in the specialty;
- protocols of the departmental council for the preliminary discussion of the pre-dissertation work and the decisions taken to disclose the procedure and composition of the scientific jury (23.02.2024)
- dissertation work;
- abstract;
- list of scientific publications on the topic of the dissertation;
- copies of the scientific publications – 3 scientific publications, 2 of them with IF and Q3 (in 2 of them the PhD student is the first author);
- list of participations in scientific forums – 6 participations in International and National scientific conferences (in 4 of them the PhD student is the first author);
- list of noticed citations – 6 citations of two of the scientific publications;
- certificate of completion of studies at the PhD School of MU-Plovdiv;
- declaration of originality and authenticity of the attached documents.

All applied documents are in full compliance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation and the Rules of the Medical University - Plovdiv.

2. Relevance of the dissertation topic

In the dissertation submitted for my opinion by assistant professor Maria Atanasova Choneva - PhD student in the Department of "Medical Biochemistry", Faculty of Pharmacy, MU-Plovdiv, a current problem is studied both in scientific and scientific-applied terms, related to research and analysis of the effects of two types of prebiotic oligosaccharides - xylooligosaccharides (XOS) and galactooligosaccharides (GOS) as well as aerobic training on metabolic and behavioral disorders in rats with streptozotocin-induced diabetes. In recent years, it has been established that the composition and metabolic function of the gut microbiota are essential for maintaining and regulating glucose levels, as well as modulating the immune system. Therefore, the study of the influence of prebiotic oligosaccharides and aerobic training on an animal model of healthy and diabetic type 1 rats expands the knowledge of their effects with a view to their possible application in clinical practice and the prevention of type 1 diabetes, which is among the leading causes of morbidity, and in the absence of adequate therapy - for high mortality worldwide.

3. Subject knowledge

After reading the dissertation work, the author's abstract and the scientific publications of assistant professor Maria Choneva, I can state that the PhD student is very well acquainted with the analyzed scientific problem, appreciates the creative scientific research of the cited authors and can accurately and competently interpret them. The *Literature review* for the dissertation work clearly and consistently introduces to the research aim that Maria Choneva has set: to determine the effect on two prebiotic oligosaccharides - XOS and GOS, and on aerobic training, alone or in combination, on metabolic and behavioral indicators in rats with experimentally induced type 1 diabetes. To realize the added goal, the implementation of 7 main tasks is envisaged: *creation of a model of type 1 diabetes in rats by treatment with streptozotocin; monitoring the influence of XOS and GOS as well as aerobic training on the metabolism and behavior of diabetic and healthy rats; evaluation of the influence of oligosaccharides and aerobic training on blood glucose status; assessment of the influence of oligosaccharides and aerobic training on animal growth by measuring somatometric indicators at the beginning and at the end of the experiment; assessment of behavior and learning and memory processes in three of the diabetic rat groups and one of the control groups; determination of microbial number and main aerobic and anaerobic microorganisms in faecal samples at the beginning and at the end of the experiment in all animals; assessment of list markers, oxidative stress and serum lipid profile at the end of the experiment in all tested animals.*

4. Research methodology

Selected research methods: *induction of type 1 diabetes after administration of streptozotocin; treatment of experimental animals with oligosaccharides; carrying out aerobic training of the animals; determination of blood glucose levels and somatometric indicators (body weight, naso-anal length, body mass index and Lee's index), as well as conducting and evaluating behavioral tests (to assess motor activity, passive learning and*

memory, anxiety index , recognition and spatial memory) are completely adequate and realistic to achieve the aim and tasks set in the dissertation work. In addition, precise biochemical, microbiological and statistical analyzes were carried out, which enriched the obtained results.

5. Characterization and evaluation of the dissertation work and contributions

The dissertation work of assistant professor Maria Atanasova Choneva contains 148 pages and is illustrated with 4 tables, 26 figures and 9 photographs. 307 literary sources are cited, most of them from recent years.

The *Literature review* is logically structured, with 32 pages describing the influence of environmental factors, symptoms, diagnosis, complications and therapy of the autoimmune disease type 1 diabetes; the composition and role of the gut microbiota and the risk of developing type 1 diabetes; the effects of probiotics on the formation of the gut microbiota and the development of type 1 diabetes, as well as the positive impact of aerobic training on immunity and gut microbiota, metabolic, cognitive and behavioral disorders. Numerous scientific studies on the subject of the dissertation are cited and analyzed in the *Literary review*, which makes an excellent impression. The aim of the dissertation work is clearly formulated, and the tasks set for implementation (described above) summarize the guidelines for performing and analyzing the intended experiments.

In the *Results and discussion* section, over 52 pages, results from precise and highly informative experiments are shown and analyzed: changes in blood glucose levels, somatometric indicators, introduced markers, lipid profile, assessment of oxidative stress, reduced indicators and microbiota analysis. The results in the dissertation are statistically analyzed and illustrated with appropriate figures.

Based on a study conducted on the individual and combined effects of the used oligosaccharides and aerobic training on metabolic and behavioral disorders and the composition of the microbiota in rats with type 1 diabetes, 7 important conclusions were summarized and 3 scientific and 3 scientific-applied contributions were formulated. The antihyperglycemic, antihyperlipidemic and antioxidant potential of the tested synthetic oligosaccharides, which also have a positive effect on states of anxiety, depression and neurocognitive disorders, has been proven. In addition, the oligosaccharides XOS and GOS can be included in the composition of synbiotics and other nutritional supplements with a beneficial effect on the growth and metabolism of the red microbiota.

The obtained results and the formulated conclusions in the dissertation work have a real application in clinical practice for the development of an adequate complex therapeutic strategy for the prevention and treatment of patients with type 1 diabetes.

6. Evaluation of the publications and personal contribution of the PhD student

The results of the dissertation have been published in 3 scientific articles in the period 2020-2022, two of them in journals with an impact factor and Q3. The total impact factor of the articles is 4.123, and the PhD student is the first author in two of the publications, which is proof of her leading role in the implementation of the experiments in the dissertation work. So far, 6 citations of the publications on the topic of the dissertation have been noticed. The results were reported at 6 International and National Scientific Forums in the period 2020-2022, with Maria Choneva being the first author in 4 of them.

I have some critical remarks, recommendations and questions about the dissertation.

1. The use of too many abbreviations in the text makes the dissertation work difficult to read and analyze.
2. I notice some inaccuracies in the statement, such as "small and large intestines" instead of "small intestine and large intestine"; etc.
3. Do you plan to conduct similar studies with diabetic animals subjected to aerobic training and a standard diet + galactooligosaccharides (DT-GOS group)?
4. Why some of analyzes (lipid profile, inflammatory markers, assessment of oxidative stress) were performed only at the end of the experiment? It would be much more precise to determine these parameters over time, as is done with the glucose levels presented in Figure 7.
5. How would you explain the identical levels of TAG (presented in Figure 10) in the two groups of healthy animals (H-XOS and H-GOS) and the diabetic animals?
6. Why significant differences in malondialdehyde levels were observed in the tested groups of healthy animals (H-XOS and H-GOS) presented in Figure 14?

In conclusion, the dissertation clearly shows that the doctoral student Maria Atanasova Choneva **possesses** in-depth theoretical knowledge and professional skills in the field of medical biochemistry, **demonstrating** qualities and skills for independent conduct of scientific research.

7. Author's summary of dissertation

The presented author's summary of the dissertation work of assistant professor Maria Choneva is perfectly designed and in accordance with the legal requirements, it fully corresponds to the content of the dissertation and provides comprehensive information about the experiments, the obtained results, discussion and analysis of the conducted research.

CONCLUSION

From the scientific and research work carried out, the presented dissertation work, abstract, scientific publications, citations and participation in International and National conferences, I can confidently summarize that assistant professor Maria Choneva is an young scientist who can plan and conduct in-depth scientific research, analyze and discuss the obtained results, as well as compare them with published scientific articles. With the presented publications and participation in scientific forums, she meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Rules for its implementation and the Rules of the Medical University - Plovdiv.

All this gives me reason to recommend to the respected members of the Scientific Jury to vote positively for the awarding of the educational and scientific PhD degree to the PhD student Maria Atanasova Choneva in the Higher Education Department: 4. "Natural Sciences, Mathematics and Informatics", Professional Direction 4.3. "Biological Sciences", doctoral program "Medical Biochemistry".

20.04.2024 **Author of opinion**:

prof. Albena Jordanova, PhD