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REVIEW of the Doctoral Dissertation

The review of the doctoral dissertation of Iskandar Azmy Harahap, M.Sc.

entitled "The study on the impact of isoflavones and probiotics on calcium bioaccessibility and calcium status – *in vitro* and *in vivo* studies"

„Badanie wpływu izoflawonów i probiotyków na biodostępność i gospodarkę wapnia – badania *in vitro* i *in vivo*"

conducted at the Department of Human Nutrition and Dietetics, Faculty of Food Science and Nutrition, Poznań University of Life Sciences

under the supervision of Prof. Joanna Suliburska, PhD, DSc

1. Formal Basis for Preparing the Review

The formal basis for preparing the review is a letter from the Chair of the Scientific Council of the Discipline of Food Technology and Nutrition, Prof. Dorota Cais-Sokolińska, PhD, from the Poznań University of Life Sciences (No. NZDT-4000-24/2024) dated October 31, 2024, informing about the resolution of the Scientific Council and entrusting me with the duties of a reviewer, and thus preparing an evaluation of the doctoral dissertation of Iskandar Azmy Harahap, M.Sc. The review was prepared based on the statutory requirements specified in the Act of July 20, 2018, Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended).

2. Presentation of Basic Information about the Candidate

Iskandar Azmy Harahap, M.Sc. obtained his master's degree in 2016 from the Department of Food Science and Technology at Gadjah Mada University in Indonesia, and then began working at the Indonesian Institute of Sciences (LIPI) as a research scientist. As a junior research scientist at this institution, he received many prestigious research grants, both domestically and internationally. In 2020, to further develop his scientific career, he began

doctoral studies at the Department of Human Nutrition and Dietetics, Faculty of Food Science and Nutrition at the Poznań University of Life Sciences in Poland under the supervision of Professor Joanna Suliburska. Iskandar Azmy Harahap M.Sc is a co-author of 26 scientific publications, being the first author in 16 of them and the corresponding author in 2, as well as a co-author of 4 chapters in monographs. Iskandar Azmy Harahap M.Sc is also a co-author of 4 patent applications (1 in Poland, 3 in Indonesia) and a beneficiary of 8 national and international research grants. It is worth noting that Iskandar Azmy Harahap M.Sc was the first laureate of the Young Scientist program at the Faculty of Food Science and Nutrition at the Poznań University of Life Sciences. In 2021, he was among the top five laureates of the Preludium grant funded by the Polish National Science Centre. Additionally, in the same year, he received a doctoral scholarship from the Nutricia Foundation, being the only PhD student to receive this grant in Poland. Moreover, Iskandar Azmy Harahap M.Sc participated in several international internships, including a 3-month Erasmus+ doctoral internship at the University of Porto in Portugal in 2021, and a 7-month internship at Leibniz University Hannover in Germany in 2024. He actively participated in 12 scientific conferences in various countries, presenting research both in the form of presentations and posters. In 2024, during the International Conference of Food Digestion in Portugal, he was a finalist for the Young Scientist Award. Additionally, in 2024, he received the Travel Grant Award and was invited to give a lecture at the International Scientific Association for Probiotics and Prebiotics (ISAPP) in Ireland, which is undoubtedly a recognition of the PhD student's scientific work. As part of his international scientific activity, Ikandar Azmy Harahap M.Sc also demonstrates his engagement as a reviewer of manuscripts submitted to high-impact journals from the JCR database (e.g., PloS One, Current Medical Science, Obesity Reviews). His current Hirsch index is 9, and the number of citations, according to the Scopus database, is 205.

3. Subject of the Review – Formal and Substantive Evaluation of the Doctoral Dissertation

The subject of the review is the dissertation titled "Study of the impact of isoflavones and probiotics on calcium bioavailability and metabolism – *in vitro* and *in vivo* studies," written under the supervision of Professor Joanna Suliburska. The doctoral dissertation of Iskandar Azmy Harahap M.Sc consists of a collection of five thematically coherent publications, which were published between 2021 and 2024. The submitted body of works includes the following publications:

P1. Harahap, I. A. & Suliburska, J. (2021). Probiotics and Isoflavones as a Promising Therapeutic for Calcium Status and Bone Health: A Narrative Review. *Foods*, 10(11), 2685–2685.

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P2. Harahap, I. A., Olejnik, A., Kowalska, K., & Sulibursk a, J. (2024). Effects of Daidzein, Tempeh, and a Probiotic Digested in an Artificial Gastrointestinal Tract on Calcium Deposition in Human Osteoblast-like Saos-2 Cells. *International Journal of Molecular Sci.* 25(1008–1008).

P3. Harahap, I. A., Kuligowski, M., Cieslak, A., Kołodziejski, P. A., & Sulibursk a, J. (2024). Effect of Tempeh and Daidzein on Calcium Status, Calcium Transporters, and Bone Metabolism Biomarkers in Ovariectomized Rats. *Nutrients*, 16(5), 651– 651.

P4. Harahap, I. A., Schmidt, M., Pruszyńska-Oszmałek, E., Sassek, M., & Sulibursk a, J. (2024). Impact of Lactobacillus acidophilus and its Combination with Isoflavone Products on Calcium Status, Calcium Transporters, and Bone Metabolism Biomarkers in a Post-menopausal Osteoporotic Rat Model. *Nutrients*, 16(15), 2524–2524.

P5. Harahap, I. A., Moszak, M., Czlapka-Matyasik, M., Skrypnik, K., Bogdański, P., & Sulibursk a, J. (2024). Effects of Daily Probiotic Supplementation with Lactobacillus acidophilus on Calcium Status, Bone Metabolism Biomarkers, and Bone Mineral Density in Postmenopausal Women: A Controlled and Randomized Clinical Study. *Frontiers in Nutrition*, 11, 1401920.

The publications included in the cycle are well-prepared and undoubtedly contribute new scientific knowledge to the field of medical and health sciences. The total impact factor (IF) of the indicated works, according to the JCR database, is 23.2, which equals 560 points according to the Ministry of Science and Higher Education (MNiSW, Poland) (as per the announcement of the Minister of Science dated January 5, 2024, regarding the list of scientific journals and reviewed materials from international conferences). The cycle of scientific publications was submitted for evaluation along with the author's summary. Considering that all the publications included in the dissertation were carefully prepared and positively reviewed by reviewers during the publication process, and were published in very good scientific journals with a wide international reach and high impact factor, I will focus on the formal and substantive evaluation of the prepared dissertation. The author's summary of the dissertation begins with an introduction, which is very important from the perspective of justifying the research topic within the chosen discipline. The way the introduction is prepared demonstrates that the PhD student has the ability to critically analyze scientific literature and plan research, with appropriate citation of sources. In the following subsections, the PhD student formulates the main theses and research objectives. The main objective of this dissertation, as defined by the PhD student, is to investigate the impact of isoflavones and probiotics on increasing calcium bioavailability and supporting bone health, particularly in the context of postmenopausal osteoporosis, combining *in vitro* and *in vivo* studies. The author then justifies the selection of publications for the cycle submitted as a scientific achievement. Next, the author justifies and describes the research methods used

during the doctoral research. The results obtained in the research are presented and analyzed, assessing their significance for the scientific field. In the summary chapter concerning research results, the author describes the subsequent publications selected for the cycle [P1-P5], linking them with the specific research objectives. The work [P1] is a review paper that compiles the most important information about nutrients with therapeutic effects, with particular emphasis on probiotics and isoflavones in the context of controlling calcium absorption and improving bone health. The remaining papers [P2 to P5] are co-authored original research articles. In the first stage of research [P2], an *in vitro* experiment was conducted using Caco-2 and Saos-2 cells to simulate human digestion and osteoblast activity in the bone mineralization process. These studies provided valuable information on the diverse effects of daidzein, tempeh, and L. acidophilus probiotics on calcium bioavailability and deposition in hematopoietic cells-osteoblasts. The next two papers [P3 and P4] involved *in vivo* studies using an OVX rat model to describe the mechanisms underlying health issues occurring during menopause. This model used female rats that had their ovaries surgically removed to induce a hormonal effect similar to menopause in women. The research was divided into two parts. In the first part [P3], the effects of pure daidzein and tempeh on calcium status, calcium transporters, and bone metabolism in rats were studied, while in the second part [P4], the therapeutic effects of *L. acidophilus* probiotics and their combination with isoflavone products such as daidzein and tempeh were evaluated. The obtained research results suggest an interdependence between dietary components, calcium metabolism, and bone health during menopause. The last paper [P5] included by the PhD candidate in the cycle of publications forming the basis of the doctoral dissertation concerns clinical studies involving postmenopausal women. This work significantly expands the scope of research conducted by Iskandar Azmy Harahap, M.Sc., as it integrates the results of laboratory and preclinical studies with clinical interventions, giving it particular scientific significance. The dissertation also includes numerous research diagrams in the form of schemas, which positively influences the reception of the work. In the summary of the dissertation, Iskandar Azmy Harahap, M.Sc. emphasizes the interconnections between the individual publications, proving that they constitute a comprehensive scientific achievement. An important element of the dissertation is the chapter on limitations and future research perspectives. Including limitations shows that the PhD candidate can critically assess their work, which is crucial in science, while indicating perspectives can inspire further research that may overcome these limitations, aiding the development of the given scientific field. Subsequent chapters include information on research funding, references, a list of tables and figures, and statements from the Bioethics Committee.

In the case of a doctoral dissertation based on a series of co-authored publications, an essential element of the review is the assessment of the PhD candidate's individual contribution to the publications comprising the dissertation. According to the requirements, statements from all co-authors of the publications and the PhD candidate himself were attached to the dissertation, definitely confirming that Iskandar Azmy Harahap's M.Sc. contribution to conducting the research and preparing all publications was significant. The individual contribution to the preparation of the publications included, among other things, the conceptualization of the research, its implementation, data processing and visualization, and preparing the publications for print.

4. Key Scientific Achievements of the PhD Candidate

The research subject undertaken by Iskandar Azmy Harahap, M.Sc., is very interesting and important, both from a cognitive and practical perspective. A notable strength of this work is its comprehensive approach to the research topic. The dissertation integrates *in vitro*, *in vivo,* and human clinical studies, offering a holistic view of the potential benefits and limitations of isoflavones and probiotics concerning calcium bioavailability and bone health. This methodological diversity enhances the robustness and credibility of the findings.

The research conducted by the PhD candidate on the combined effects of daidzein and tempeh with probiotics, such as *L. acidophilus*, on calcium metabolism and bone health, represents one of the first systematic and comprehensive studies presented in the literature. The use of an artificial gastrointestinal model to study digestion and the subsequent impact on calcium deposition in osteoblastic cells is a particularly novel aspect, providing clear insights into the bioactive potential of these compounds post-digestion. The results obtained by the PhD candidate in studies conducted on OVX rat models are also significant. These studies provide evidence suggesting that products rich in isoflavones, such as tempeh and daidzein, in combination with probiotics, may serve as viable alternatives or supplements to conventional osteoporosis treatments. Crucial in the context of the PhD candidate's research is the clinical study involving postmenopausal women. The finding that daily supplementation with *L. acidophilus* helps stabilize bone turnover, despite slight changes in bone mineral density profiles, forms the basis for future research and potential dietary recommendations for postmenopausal women.

The obtained results indicate potentially beneficial dietary interventions for the treatment of menopausal osteoporosis, offering practical solutions that can be implemented in real-world scenarios. The dissertation integrates multiple scientific disciplines, and such an interdisciplinary approach enriches the study and broadens its application possibilities.

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The innovative approach of the PhD candidate significantly contributes to the fields of food and nutrition sciences as well as medical sciences. Additionally, the work outlines future research perspectives, which are crucial for the advancement of science.

5. Detailed Remarks and Discussion

From both a formal and substantive perspective, I evaluate the research conducted by the PhD candidate very positively. The doctoral dissertation merits a high rating, particularly due to the exceptional thematic coherence of the publication cycle. It is worth noting that the presented studies exhibit significant interconnections, with each subsequent work continuing the previous stage. This structure allows for a comprehensive understanding of the impact of isoflavones and probiotics on calcium bioavailability and bone health in various experimental models, including *in vitro* experiments, animal studies, and human clinical trials.

When familiarizing myself with the content of the dissertation, a few general questions arise:

• When choosing a supplement preparation, the chemical form of the element is an important consideration. In your research, you used calcium citrate. On what basis was this chemical form of calcium selected? Did you consider using other forms that contain a higher amount of elemental calcium form a chemical standpoint?

• Literature indicates that many factors influence calcium absorption, not just vitamins D and K, which you mentioned in the limitations of your publications [P2, P3]. The weight ratio of calcium to phosphorus is also important. In your publications [P2, P4], you indicate that calcium bioavailability in the digestive system does not necessarily correlate with its deposition in bone cells/bones. Therefore, the question arises as to whether you controlled the calcium to phosphorus ratio in your *in vitro* and *in vivo* studies. The appropriate ratio of these elements also affects their mutual absorption and utilization by the body.

• In quantitative studies, particularly those concerning calcium determination, obtaining reliable results is crucial. In your works, you establish the measurement traceability by using certified reference materials and provide the trueness of the applied analytical procedure (which is always 92% for various CRMs). However, none of the publications include information on the quality control parameters of the measurement results assessed during the validation of the applied analytical method. Did you determine other validation parameters of the applied procedure, such as detection limit, quantification limit, measurement range, measurement precision?

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6. Summary of the Review

In conclusion, I affirm that the doctoral dissertation of Iskandar Azmy Harahap, M.Sc., is a carefully planned and executed study on the potential benefits of isoflavones and probiotics for calcium bioavailability and metabolism in the body. The comprehensive approach, innovative insights, and practical implications make the dissertation a significant contribution to the field of medical and health sciences, paving the way for future research and potential clinical applications. **With full conviction, I state that the doctoral dissertation meets the statutory requirements set for candidates for the doctoral degree, as specified in the Act of July 20, 2018 – Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended). Based on this, I am fully convinced and hereby propose to the Discipline Council of the Faculty of Food Technology and Nutrition of the Poznań University of Life Sciences that Iskandar Azmy Harahap, M.Sc., be admitted to the further stages of the doctoral procedure.**

Furthermore, I respectfully propose that the doctoral dissertation of Iskandar Azmy Harahap, M.Sc., be considered for distinction, and the relevant document with justification is attached to the review.

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APPLICATION FOR DOCTORAL DISSERTATION DISTINCTION

I hereby submit a request to the Discipline Council of the Faculty of Food Technology and Nutrition at the Poznań University of Life Sciences for the distinction of the doctoral dissertation by Iskandar Azmy Harahap, M.Sc., entitled "Study of the effects of isoflavones and probiotics on calcium bioavailability and metabolism – *in vitro* and *in vivo* studies," conducted at the Department of Human Nutrition and Dietetics at the Faculty of Food Science and Nutrition, Poznań University of Life Sciences, under the supervision of Professor Joanna Suliburska.

I highly evaluate the overall substantive value of the dissertation presented for my assessment. I consider this doctoral dissertation to be highly novel and original, significantly exceeding the average level of doctoral dissertations in terms of research quality and scope. The research is unique in its analysis of the synergistic effects between isoflavones and probiotics and the evaluation of the potential practical applications of the obtained results in the treatment of postmenopausal osteoporosis. The conducted studies demonstrate that isoflavone-rich products, such as tempeh and daidzein, in combination with probiotics, can be an alternative or complement to conventional osteoporosis treatments. The comprehensive nature of the research conducted by the PhD candidate, including in vitro, in vivo, and human studies, provides a holistic view of calcium bioavailability and bone health. The obtained results provide practical solutions that can be implemented in real-world scenarios and undoubtedly offer valuable insights in both the fields of food science and nutrition and medical sciences.

The innovative nature of the research conducted by the PhD candidate and the obtained results are demonstrated by both the scientific publications that form the basis of the doctoral dissertation (1 review paper and 4 original papers) and an additional 21 publications co-authored by Iskandar Azmy Harahap, M.Sc. All papers are published in renowned journals from the JCR list, with high impact factors and have already been frequently cited, which most clearly attests to their high scientific value.

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