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REVIEW

of the doctoral dissertation submitted by **mgr Bukola Muibad Adenuga** entitled: **Authentication of selected game species in food products based on nuclear markers and qPCR** carried out at the Department of Meat Technology, Faculty of Food Science and Nutrition, Poznań University of Life Sciences under the supervision of prof. dr hab. Magdalena Montowska

Basis for the Preparation of the Review

The basis for the preparation of this review is the letter dated 12 December 2025 (NZDT-4000-5/2025) from the Chair of the Scientific Council of the Discipline of Food Technology and Nutrition at the Poznań University of Life Sciences, Prof. UPP Dr. Hab. Dorota Cais-Sokolińska. The review has been prepared in accordance with the provisions of the Act of 20 July 2018 – Law on Higher Education and Science (consolidated text: Journal of Laws of 2024, item 1571, as amended), as well as the relevant implementing regulations governing the procedure for awarding the doctoral degree, including the requirements concerning the evaluation of the doctoral candidate's scientific achievements, the substantive value of the dissertation, and the criteria specified in Article 186 of the Act.

Justification for Undertaking the Research Topic

Taking into account the above legal framework, I hereby present my evaluation of the doctoral dissertation, considering its originality, methodological soundness, the significance of the research results obtained, and their contribution to the development of the discipline.

The decision to address the development and validation of sensitive, specific, and reproducible real-time quantitative PCR (qPCR) methods for the identification and quantification of the three most popular game animal species—red deer, roe deer, and wild boar/domestic pig—is fully justified from both scientific and practical perspectives. The

European game meat market is growing dynamically, and consumers increasingly expect high-market-value products originating from legal and sustainable sources, which heightens the need to ensure their authenticity.

The determination of the species origin of processed food components and their raw materials is becoming increasingly important for both producers and consumers. Growing concern for food quality and its beneficial impact on human health has led to greater emphasis on reliable information regarding the actual composition of food products. Economic considerations also play a significant role, as they may encourage some producers to engage in deliberate adulteration, particularly by substituting higher-market-value meat with cheaper, usually lower-quality raw materials. Moreover, the number of consumers seeking distinctive food products—such as game meat, exotic foods including seafood, or products with specific nutritional properties—is steadily increasing.

It should also be emphasized that game meat is particularly vulnerable to fraud, including species substitution, mislabeling, mixing with meat from other animals, or illegal trade in protected species. Traditional species identification methods based on morphological or protein characteristics often prove unreliable in the case of processed products. The application of qPCR enables precise, reproducible, and quantitative detection of species-specific genetic material, even in complex biological matrices subjected to thermal processing. The development of such methods is of significant scientific value and may support official food control authorities as well as producers in ensuring legal and transparent meat trade, while addressing the real needs of the European market, where red deer, roe deer, and wild boar are among the most frequently marketed game species.

Formal and substantive evaluation of the dissertation

The doctoral dissertation submitted for review comprises a 206-page study presenting a thematically coherent series of five scientific publications. The structure of the dissertation meets the formal requirements and complies with the editorial standards applicable to doctoral theses. The section preceding the table of contents includes a list of publications forming the series (complete bibliographic data, MNiSW scores, and Impact Factor values according to the year of publication), as well as abstracts in both English and Polish.

The structure of the dissertation is typical of experimental research and consists of nine chapters: Introduction (2 pages), Rationale of the Study (4 pages), Research Hypothesis and Objectives (1 page), Materials and Methods (5 pages), Results and Discussion (15 pages), Conclusions (2 pages), and References (7 pages), preceded by Abstracts in Polish (2 pages)

and English (2 pages), a title page, and a table of contents. The dissertation also includes Authors' Statements regarding the publications (10 pages), copies of the scientific articles constituting the core achievement (161 pages), supplementary materials (3 pages), and statements by the doctoral supervisor (1 page) and the doctoral candidate (1 page) which constitute a technical supplement to the dissertation, as required by the applicable regulations. The structure of the dissertation is logical and transparent. All elements were prepared correctly, with a high level of editorial diligence and in accordance with the principles of scientific documentation. The experimental section has been divided into subsections titles of which concisely reflect their respective contents. The dissertation has been prepared with due diligence and it demonstrates the Candidate's strong familiarity with the subject matter.

The doctoral candidate formulated the main objective of the dissertation, which was the development and validation of sensitive, specific, and reproducible quantitative real-time PCR (qPCR) methods for the identification and quantification of three common game species in Poland (red deer, roe deer, and wild boar/domestic pig), which are also popular on the European game meat market. To verify these research assumptions, specific objectives were defined and subsequently achieved, with the results presented in three of the five research publications (P3–P5). The first two publications (P1, P2) constitute literature reviews typical for a doctoral dissertation.

In P1, the author provides a comprehensive analysis of the Nigerian meat industry, examines selected cases of meat fraud, and discusses authentication methods applied in neighboring African countries, offering valuable insights for Nigerian researchers and national regulatory authorities. It is, however, unfortunate that in this part of the work, the doctoral candidate did not provide neither a detailed characterization of game meat consumption in Nigeria compared to European countries, nor a comprehensive market analysis including, for example, production volume, species, sources of origin, seasonality, consumption levels, market value, and specific health-related properties of game meat. Although this was not the primary focus of the dissertation, in the reviewer's opinion, including such information could have enriched the literature review by illustrating the quantitative scale of the issue under discussion.

In the subsequent literature review (P2), the doctoral candidate presented various DNA-based analytical methods used by researchers to identify and quantitatively assess meat from game species and uncommon wildlife, such as monkeys, crocodiles, llamas, bison, and others. Although polymerase chain reaction (PCR) is considered the standard and recommended DNA analysis technique for meat authenticity studies, it is noteworthy that the doctoral

candidate also described unconventional PCR-based methods in the article, such as Fluorescence Resonance Energy Transfer (FRET) techniques, Low-Cost and Low-Density Array (LCDA) technology, PCR-based lateral flow immunoassays (LFI), and DNA strips, as well as non-PCR methods, including Loop-Mediated Isothermal Amplification (LAMP), Recombinase Polymerase Amplification (RPA), Cross-Priming Amplification (CPA), and Nanopore sequencing technology, among others.

For the preparation of the literature review, the author selected 131 articles based on criteria such as the species of the studied animal, the analytical method used, and the year of publication. The results highlight the most frequently authenticated game and exotic species, PCR- and alternative-based analytical methods, and the potential applications of molecular techniques in wildlife conservation.

The dissertation was prepared with due diligence and demonstrates the Candidate's strong understanding of the subject matter addressed. The main objective of the dissertation, supported by six specific objectives, has been precisely defined and thoroughly justified through the presentation of its rationale and its grounding in the current state of knowledge. The research hypothesis was formulated clearly and unambiguously by the Candidate, remaining closely aligned with the subject of the study. It is empirically verifiable, which enabled the proper design of the research and ensured a consistent analytical focus on identifying and evaluating relationships among the examined factors.

Noteworthy is the logical and fully justified structure of the dissertation, which clearly highlights the scope of the research and analyses conducted. This approach effectively guides the experimental work, facilitating the achievement of the main research objective and thereby ensuring the resolution of the research problem.

In the chapter "Materials and Methods," the PhD Candidate characterized the research samples obtained from hunting district No. 57, the Sarbia Forest District in Greater Poland, specialty game meat shops, local shops and supermarkets in Poznań (Poland), local shops in Portugal, and online retailers in France and Spain. The Candidate included a diverse set of samples in the study, classified as follows: target species samples, non-target species samples, plant-derived ingredients used in production processes, 57 diversified game meat products, and model meat products produced under laboratory conditions with a detailed description of the production process.

Subsequently, the Candidate presented the methodology for primer design, verification of specificity both in silico and in vitro, and optimization of qPCR conditions. The chapter is characterized by a clear presentation of TaqMan probe-based procedures, ensuring

reproducibility and enabling precise detection and quantification of red deer, roe deer, wild boar, or pork meat in processed food products. It is noteworthy, that all assays demonstrated high analytical sensitivity, with limits of detection (LOD) and quantification (LOQ) at the level of 0.01 ng for the tested species. The quantitative matrix-based method enabled the Candidate to achieve relative detection limits as low as 0.5% for red deer meat, 0.1% for wild boar, and 0.05% for roe deer in the designed meat compositions. Cross-reactivity tests using 16 animal species, including rare and farmed species, as well as various herbs and spices, confirmed the high specificity of the assays. Validation experiments performed with laboratory-prepared meat mixtures subjected to different processing conditions (raw meat, baking, and sterilization) further highlighted the robustness of the method. The proposed procedure demonstrates a professional methodological approach conducive to effectively solving complex research problems.

In the subsequent chapter of the dissertation, "Discussion and Interpretation of Results," the Candidate presented and discussed the findings obtained through the consistent implementation of the research objective in accordance with the adopted methodology and staged structure. This structure results, among other factors, from the publication of the research findings as manuscripts forming a series corresponding to the topic of the evaluated doctoral dissertation.

Following successful validation of the species-specific qPCR methods developed by the Candidate, commercial game meat products were analysed to assess labelling accuracy and detect potential adulteration. A total of 58 products were tested in three studies focusing on red deer, roe deer, and wild boar/pork content. The samples originated from Poland, Spain, Portugal, and France, and included a wide range of processed products such as sausages, pâtés, loins, terrines, and canned meats.

In the red deer study (P3), the Candidate found that among the 28 products labelled as containing red deer meat, 13 showed a substantial reduction or complete absence of this species. In some cases, the red deer content was more than ten times lower than declared. Moreover, undeclared red deer meat was detected in seven of the 14 products labelled as containing roe deer and in both products labelled as fallow deer.

In the roe deer study (P4), the Candidate reported that of the 14 samples labelled as containing roe deer meat, only nine tested positive for this species. Six samples showed total species substitution, while eight exhibited a significant reduction in roe deer content. Roe deer DNA was also detected in eight products labelled as containing red deer meat and in one product labelled as fallow deer, indicating undeclared addition. The combined analysis of roe

and red deer results revealed widespread mislabelling, with as many as 61% of the analysed products being adulterated.

In the wild boar/pork study (P5), varying levels of agreement between declared and determined species content were demonstrated. Four products labelled as containing only deer species tested positive for wild boar/pork, and in some cases the detected levels suggested intentional adulteration.

The results obtained by the Candidate and published in papers P3, P4, and P5 indicate systemic problems in the game meat sector, where intentional mislabelling and species substitution are widespread. The findings emphasize the need for routine application of quantitative molecular methods to ensure food authenticity and protect consumer rights.

The Candidate's experience in scientific writing is demonstrated by the reliable discussion of the obtained results in reference to findings reported by other authors working on similar research topics. This reflects the Candidate's scientific maturity and responsibility.

The research results presented in five publications enabled the Candidate to formulate seven conclusions, included in the chapter "Conclusions." Each of these conclusions aligns with the stated objectives of the study. In the reviewer's opinion, their number is appropriate to the scope of the conducted research and corresponds to the volume of data obtained.

In the dissertation submitted for evaluation, the Candidate cited 80 references (with an even broader literature base included in the publications forming part of the achievement), appropriately selected in relation to the scope and subject of the research. These references are English-language publications from the last decade, predominantly scientific articles addressing the discussed issues, which confirms the innovative character of the work.

At the same time, I ask the Doctoral Candidate to address the following discussion questions:

1. How do the quality and source of DNA (raw plant materials, raw animal materials, processed products) affect the reliability of detecting selected species using the PCR method?
2. What are the advantages and disadvantages of using nuclear markers compared to mitochondrial markers for quantitative species determination in food authentication?
3. What are the limitations of qPCR tests in detecting very low levels of DNA, and how this might influence regulatory decisions (e.g., regarding compliance with standards or interpretation of control results)?
4. Could the methodology applied in the doctoral dissertation be implemented for authenticating other species, such as fish or exotic animal meats?

5. How can repeated inaccuracies in species labeling of meat products shape consumer perceptions of the safety and quality of game meat?

In summary, the dissertation presented by the Candidate is written in correct and precise scientific language. It includes properly placed references to the literature and clearly delineates content related to the individual articles forming the publication series. This way of organising the presentation of material significantly facilitates tracking the course of the research and allows for a more comprehensive understanding of its scope and results.

The publications included in the doctoral dissertation were published in the years 2023–2025. They are as follows:

P1. Adenuga, B. M., Montowska, M. (2023). The Nigerian meat industry: An overview of products' market, fraud situations, and potential ways out. Acta Scientiarum Polonorum Technologia Alimentaria, 22(3), 305-329. http://dx.doi.org/10.17306/J.AFS.2023.1157 MNiSW = 40 points, IF = 1.30

P2. Adenuga, B. M., Montowska, M. (2023). A systematic review of DNA-based methods in authentication of game and less common meat species. Comprehensive Reviews in Food Science and Food Safety, 22, 2112-2160. https://doi.org/10.1111/1541- 4337.13142 MNiSW = 200 points, IF = 12.00

P3. Adenuga, B. M., Biltes, R., Villa, C., Costa, J., Spychaj, A., Montowska, M., Mafra, I. (2025). Unravelling red deer (Cervus elaphus) meat adulteration in gourmet foods by quantitative real-time PCR. Food Control, 168, 110872. https://doi.org/10.1016/j.foodcont.2024.110872 MNiSW = 140 points, IF = 5.60

P4. Adenuga, B. M., Biltes, R., Villa, C., Costa, J., Spychaj, A., Montowska, M., Mafra, I. (2024). A novel normalized quantitative real-time PCR approach for ensuring roe deer (Capreolus capreolus) meat authenticity in game meat foods. Foods, 13(23), 3728. https://doi.org/10.3390/foods13233728 MNiSW = 100 points, IF = 4.70

P5. Adenuga, B. M., Spychaj. A, Montowska, M. (2025). A new nuclear marker for quantitative analysis of wild boar and domestic pig meat in game meat products using PLAG1 zinc finger gene. Scientific Reports, 15, 20454. https://doi.org/10.1038/s41598-025-05167-x MNiSW = 140 points, IF = 3.80

Total points according to the MNiSW: 620 Total Impact Factor: 27.40

The presented doctoral dissertation entitled "Authentication of Selected Game Species in Food Products Based on Nuclear Markers and qPCR," prepared in the form of a series of five publications, meets all formal requirements described for this type of work. The body of work is thematically coherent and corresponds to current research trends in the field of food science

and nutrition. The co-authors' statements allow for a clear identification of the contribution made by M.Sc. Bukola Muibad Adenuga, who served as the first author in all five publications. Her involvement encompassed the full spectrum of research activities, including the development of the research concept and experimental design, the execution of analyses, as well as data interpretation and manuscript preparation.

In reviewing the scientific achievements of M.Sc. Bukola Muibad Adenuga, it should also be emphasized that, in addition to the five publications constituting the doctoral dissertation, the Candidate's academic record includes three additional scientific publications and five conference contributions.

The literature cited in the dissertation comprises 80 English-language bibliographic references, which confirms the Author's familiarity with and ability to effectively use international literature related to the subject of the dissertation. The inclusion of publications from the past decade demonstrates the Author's ongoing engagement with the topic and attests to the timeliness of the research problem addressed.

In conclusion, I would like to encourage the Author to exercise greater care in the future in avoiding stylistic, punctuation, and typographical errors. Furthermore, it should be noted that not all authors cited in the text are included in the reference list, and conversely, the reference list contains authors who are not cited in the main body of the dissertation.

Final Conclusion

The doctoral dissertation submitted for review constitutes a coherent, valuable, and formally complete series of publications that aligns with current research trends concerning the application of modern analytical methods in food authentication. The presented work demonstrates the Candidate's very strong substantive preparation and a high degree of research independence, confirmed by her significant and well-documented contribution to the development of the publications—from the conceptual design of the study, through the execution of the research, to the preparation of the manuscripts.

The subject matter of the dissertation falls within the field of food science and nutrition, and the manner in which it has been prepared reflects the Candidate's solid preparation for scientific research, her proficiency in modern analytical techniques, and her extensive theoretical knowledge related to the undertaken topic. Particular recognition should be given to the very appropriate selection of the research topic and the reliable implementation of successive research stages using advanced analytical methods. The results obtained by M.Sc.

Bukola Muibad Adenuga undoubtedly possess both cognitive (scientific) and practical significance.

I conclude that the doctoral dissertation entitled **"Authentication of selected game species in food products based on nuclear markers and qPCR"**, prepared by Ms. Bukola Muibad Adenuga under the supervision of prof. dr. hab. Magdalena Montowska, meets the requirements set for doctoral dissertations. Therefore, I hereby recommend to the Scientific Council of the Discipline of Food Technology and Nutrition at the Poznań University of Life Sciences that Ms. Bukola Muibad Adenuga **should be admitted** to the subsequent stages of the doctoral procedure.

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