

Review

of the doctoral dissertation submitted by MSc Bukola Muibat Adenuga entitled “Authentication of selected game species in food products based on nuclear markers and qPCR”, supervised by Professor Magdalena Montowska.

This review has been prepared in relation to the following documents:

- the letter of the Chair of the Scientific Council of the Discipline of Food Technology and Nutrition, Prof. Dorota Cais-Sokolińska, Poznań University of Life Sciences (ref. no. NZDT-4000-5/2025), who pursuant to the resolution of the Scientific Council of the Discipline of Food Technology and Nutrition of the Poznań University of Life Sciences of 11 December 2025, requested an evaluation of the abovementioned doctoral dissertation;
- the Regulation of the Minister of Science and Higher Education of 20 July 2018 (Journal of Laws 2020, item 85, as amended),
- the doctoral dissertation submitted in the field of agricultural sciences, within the discipline of Food Technology and Nutrition, submitted by MSc Bukola Muibat Adenuga entitled “Authentication of selected game animal species in food products using nuclear markers and qPCR”.

Significance of the research topic

The research issue concerning food authenticity, particularly the authentication of game meat, is of considerable importance in the context of contemporary challenges facing the food market. Authenticity refers to the consistency between declared information and the actual composition, origin, or method of production of food products, and it constitutes the foundation of trust in the producer–consumer relationship. Events such as the 2013 European “horsemeat scandal” revealed the scale of risks associated with meat product adulteration and the vulnerability of supply chains to fraudulent practices. Raw materials derived from wild animals are especially susceptible to fraud due to their high market value, niche character, and growing consumer interest in natural food products. Significant price differences between meat from farmed animals and that from wild species encourage the substitution of more expensive species with cheaper raw materials, such as pork or poultry, particularly in processed products

where species identification is more difficult. Studies conducted in various parts of the world have confirmed numerous discrepancies between product labelling and actual meat composition, leading to economic, legal, and social consequences. Consumers may suffer financial losses and unknowingly violate religious or dietary restrictions, while honest producers lose market competitiveness. An appropriate response to these challenges is the application of analytical methods for verifying species composition, particularly DNA-based techniques such as polymerase chain reaction (PCR) and real-time PCR (qPCR), which enable sensitive and specific species identification even in highly processed products. In the Polish context, there is a clear need to develop quantitative methods for precisely verifying the content of red deer, roe deer, and wild boar meat in food products. Research in this area contributes to strengthening the food control system, enhancing consumer protection, and increasing market transparency.

Formal assessment of the dissertation

The dissertation under evaluation comprises a series of five thematically related original scientific publications, published in journals included in the 2024 list of scientific journals issued by the Ministry of Science and Higher Education, namely *Acta Scientiarum Polonorum Technologia Alimentaria*, *Comprehensive Reviews in Food Science and Food Safety*, *Food Control*, *Foods*, and *Scientific Reports*. In all these publications, the Doctoral Candidate is the first author. However, the dissertation does not specify the individual co-authors' percentage contributions, which slightly limits the ability to assess their respective involvement in developing the research concept, executing the experimental work, and preparing the manuscripts. The publications are accompanied by a comprehensive study prepared by the Candidate in English, which includes the following sections: SUMMARY, STRESZCZENIE (summary in Polish), INTRODUCTION, RESEARCH HYPOTHESIS AND OBJECTIVES, MATERIALS AND METHODS, RESULTS AND INTERPRETATION, GENERAL DISCUSSION, CONCLUSIONS, REFERENCES, AUTHORSHIP CONTRIBUTION STATEMENTS and COPIES OF PUBLISHED PAPERS COMPRISING THE SERIES OF PUBLICATIONS WITH SUPPLEMENTARY MATERIALS. However, in my opinion, the Candidate should consider including a list of abbreviations used in the dissertation, as this element typically facilitates readability and consolidates the full forms of abbreviated terms in one place. It would also eliminate the need to repeatedly expand terms such as polymerase chain reaction (PCR). The manuscript (excluding the appended publications) comprises 50 pages (not including declarations and copies of the articles) and contains four tables, two figures, and 80 bibliographic references. The dissertation generally

follows the standard format for this type of academic work. In my assessment, the chapter designated as INTRODUCTION predominantly reads like a LITERATURE REVIEW. The inclusion of a more synthetic, conceptually focused INTRODUCTION section outlining the dissertation's overall framework would have enhanced the work's structural coherence. A general outline of the research problem at the beginning of the dissertation is essential for familiarising the reader with the subject matter and establishing the principal perspective of the study. Furthermore, prior to presenting the research objectives and hypotheses, a sufficiently comprehensive background should be provided to justify the necessity of conducting the studies described in the dissertation. The layout of the manuscript is clear, and the sequence of subsections within individual chapters is logical. However, the table of contents should precede the list of publications constituting the basis of the dissertation, particularly since the list of publications itself forms part of the table of contents. In summary, it may be concluded that the doctoral dissertation under review has been prepared with due diligence, which reflects the Candidate's sound editorial competence.

Scientific assessment of the dissertation

The basis for awarding the doctoral degree to MSc Bukola Muibat Adenuga is a series of five thematically related original scientific publications, published between 2023 and 2025 in peer-reviewed journals listed by the Ministry of Science and Higher Education. These publications have already undergone evaluation by independent reviewers and, in their opinion – as well as in my own – constitute valuable contributions that advance current knowledge in the field. Since the scientific merit of the individual articles has been previously assessed by their reviewers, who deemed them worthy of publication, the substantive part of my evaluation will focus primarily on the Doctoral Candidate's work.

Title of the dissertation thesis “Authentication of selected game species in food products based on nuclear markers and qPCR” concisely reflects the content contained therein; however, I would suggest replacing the phrase “based on” with “using,” with the remainder of the title adjusted accordingly. The SUMMARY is well written and contains numerous key pieces of information that familiarise the reader with, among other aspects, the study's objective, the applied methodology, the principal findings, and the conclusions. This section concludes with appropriately selected keywords. However, in my opinion, it would be advisable to avoid repeating terms already used in the title. The STRESZCZENIE (Polish-language summary) appears to be a direct translation of the English version, resulting in several linguistically awkward formulations in Polish, such as “exotic and sustainably sourced proteins” and “they were entirely absent from the declaration.” In the latter case, it would be more precise to state

that information regarding the species was absent from the declaration, rather than the species themselves. Additionally, the sentence stating that “These findings raise serious concerns regarding regulatory compliance, consumer deception, and food safety risks” would benefit from linguistic refinement to improve clarity and stylistic correctness in Polish. Furthermore, in both summaries, the Candidate does not clearly indicate which sector or stakeholder group may benefit from the solutions developed within the framework of this doctoral work.

The information presented in Chapter 1 (INTRODUCTION) appropriately and effectively outlines the issues related to the justification for undertaking the research topic. The Candidate skilfully and clearly describes the research problem concerning food authenticity, particularly in relation to game meat, which constitutes a significant and timely area of analysis in the context of contemporary challenges facing the food market. The appropriately selected and cited literature used to present the various issues addressed in this part of the dissertation deserves recognition. However, in my opinion, this section of the manuscript also contains several issues that have not received sufficient attention (likely due to its synthetic nature). For example, the Author provides only a rather cursory characterization of the challenges faced by the food sector in ensuring the species identity of marketed products. I do not concur with the statement that consumers are seeking exotic sources of protein, as no data are presented to substantiate it. This part of the dissertation also lacks quantitative data to illustrate the cited significant increase in consumer interest in meat from wild animals, as well as statistical evidence supporting the identification of France, Spain, Italy, Poland, and Germany as countries with the highest proportions of hunters. A minor shortcoming is the assertion that game meat has a more favourable chemical profile (in terms of fatty acid and cholesterol content) without specifying the actual levels of these components or providing a comparison with meat from conventional slaughter animals. In the third paragraph, the Candidate lists wild animal species in Europe of interest to the meat industry; however, for scientific accuracy, common names should be supplemented with their Latin (scientific) equivalents. This comment also applies to subsequent parts of the dissertation (e.g., the term “Sika deer”). Once both the common and scientific names have been provided, it is acceptable to use them consistently thereafter. Furthermore, I consider the placement of Figure 1 at the end of the subsection to be inappropriate, as it should appear immediately after its first reference in the text. Additionally, the figure itself presents categories of food adulteration, whereas the paragraph in which it is cited concerns the determination of species identity in food products, creating an inconsistency between the text and the illustration.

In Chapter 2 (RESEARCH HYPOTHESIS AND OBJECTIVES), the Candidate correctly formulates the hypothesis that red deer, roe deer, and wild boar contain specific DNA

sequences that can be used as nuclear markers for the authentication of food products. Furthermore, MSc Bukola Muibat Adenuga appropriately defines the principal objective of the study, namely the development and validation of real-time qPCR methods based on nuclear markers for the detection and quantification of species-specific DNA sequences of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa scrofa*) in food products. However, certain reservations arise regarding the specific objectives, which, in my opinion, should be formulated to better reflect the research's logical, sequential progression. In particular, the outcomes of earlier stages of the work should explicitly determine and guide the subsequent research approach.

Having assessed the study's methodology, I conclude that the Candidate appropriately selected and implemented all necessary laboratory and analytical methods to conduct the planned experiments. Emphasis should be placed on the broad and diverse range of research methods employed, including literature data analysis, procedures for the collection and preparation of biological samples, molecular laboratory techniques, and bioinformatics and statistical analyses. Special recognition is also due to the Candidate's advanced research competence, demonstrated in the development of standard curves that were subsequently used, *inter alia*, to assess adulteration in meat products marketed in Poland, Spain, Portugal, and France. Despite the overall methodological soundness, several shortcomings can be identified in this section. In subsection 3.1, the Candidate should explicitly specify the animal species and plant-derived additives included in the study. The information presented in this section would benefit from being organised in a tabular format, with clear divisions corresponding to subsections (a–e), thereby enhancing clarity and readability. The statement that “several” primers were designed is too general; the exact number should be provided. Moreover, the dissertation lacks detailed information regarding the criteria applied for primer and probe selection in the qPCR analyses, as well as a thorough description of the optimisation procedures for reaction conditions, analogous to the description provided for conventional PCR. In my opinion, post hoc statistical tests should also have been applied to the analysis of results to provide formal statistical confirmation of the observed differences. Additionally, this section would benefit from a clearer indication of which research components were conducted in each publication, particularly for papers P3–P5 and subsections 3.1, 3.2, and 3.3. The inclusion of schematic diagrams or graphical representations illustrating the overall research strategy could further enhance the clarity of presentation. I expect that during the public defence, this part of the dissertation will be presented in greater detail and with a more systematic structure.

In the chapter entitled RESULTS AND INTERPRETATION, the Candidate has appropriately distinguished subsections in which conscientiously presents the obtained

findings. The clear and consistent manner of data presentation deserves recognition. Nevertheless, certain shortcomings are also evident in this chapter. In my opinion, the description of the results presented in papers P1 and P2 should contain more detailed information. For example, in paper P2, the Candidate indicates contamination; however, does not specify its nature or identify its probable source. In subsequent parts of this chapter, the presentation of findings without numerical values (e.g., concentrations or quantities of DNA obtained) makes it more difficult to fully assess this section of the dissertation. Although the thematically coherent set of publications constitutes an integral part of the dissertation, I consider that the description of results in the main body of the dissertation should include more detailed information so that the reader is not required to consult the source publications repeatedly. I also consider the repeated presentation of information concerning the analysed markers to be a minor shortcoming. A detailed description of the markers and their functions should be provided earlier in the dissertation, for instance, in the chapter devoted to methodological aspects. Furthermore, the inclusion of subsection 4.2 on optimising amplification conditions and marker application strategies appears unjustified in the context of this section. Similar reservations apply to subsection 4.5.

In the subsequent chapter, GENERAL DISCUSSION, the Candidate presents numerous findings from her own research and appropriately confronts them with the results of other studies, for example, those reported by Druml et al. (2015). The dissertation confirms an alarmingly high rate of discrepancies in the declared species identity of commercially available game meat products on the European market. Mislabelling was identified in as many as 54% of products declared as red deer meat and 64% of products marketed as roe deer meat. Furthermore, undeclared pork material was detected in a portion of the analysed samples. The Author also provides a clear discussion of the newly developed TaqMan assays based on nuclear markers (TNNI2, ASIP, PLAG1) and emphasises their validation in authentic, complex food matrices, which constitutes a significant applied strength of the work. The Candidate subsequently provides a well-founded justification for selecting single-copy nuclear markers for precise quantitative analysis and appropriately discusses the advantages and limitations of mitochondrial markers. Particular recognition is due to the direct comparison of the obtained LOD and LOQ values with those reported in previous studies, which clearly situates the dissertation's achievements within the current state of knowledge. The high linearity values ($R^2 > 0.98$), low coefficients of variation ($CV < 25\%$), and validation using commercial samples confirm the high reproducibility and practical applicability of the developed method. In this part of the dissertation, the Author also logically presents the consequences of the detected irregularities for consumers, industry stakeholders, and

regulatory authorities, referring to contemporary research and examples of international food fraud scandals. Candidate accurately identifies the economic, ethical, and health-related implications of food adulteration, as well as the issue of erosion of consumer trust. Special recognition should be given to the linkage of the research findings with current EU regulations, including Regulation (EU) No 1169/2011 (FIC), which further underscores the practical dimension of the obtained results. The chapter concludes with a balanced discussion of the study's limitations, addressing both practical constraints (high costs, the need for specialised equipment and qualified personnel) and biological limitations resulting from the close genetic relatedness of the analysed species. The Candidate correctly notes that, despite the high effectiveness of the developed qPCR methods, their application under consumer-level conditions remains limited, and that alternative field-based solutions still require laboratory confirmation. Particularly commendable is the transparent discussion of reduced sensitivity observed in certain members of the Cervidae family, as well as the difficulties associated with distinguishing subspecies within Suidae. In the final subsection, the Author appropriately notes that although products from several European countries were included in the analysis, a broader geographical scope would enable a more comprehensive assessment of the transnational character of food adulteration. The recommendation to expand the panel of analysed species to include additional taxa relevant to the game meat market (e.g., fallow deer, mouflon, hare) is substantively justified and would enhance the universality of the developed method. Likewise, the proposed application of the assays in broader market monitoring appears well-founded and would enable further validation of their usefulness in regulatory and industrial practice. This section demonstrates the Author's awareness of the study's limitations and reflects a mature and forward-looking approach to the further development of the method.

In the dissertation, the Candidate formulates seven comprehensive conclusions that address the principal elements of the dissertation. These conclusions are coherent, logically structured, and clearly derived from the conducted research. The Author provides a concise synthesis of both the methodological aspects of the work, namely the development and validation of the nuclear markers TNNI2, ASIP, and PLAG1, and its practical dimension, encompassing the application of the developed assays to the analysis of commercially available products. A significant strength of the dissertation is also the summary of the market study results, which confirmed the presence of numerous irregularities in declared species composition. This finding confers clear practical and regulatory relevance upon the work. The dissertation also contains several minor linguistic and editorial errors (e.g., the omission of the publication year for the work by Hanner & Kelly), which, however, do not diminish the overall scientific value of the study and are issues that may occur even in the work

of experienced researchers. In conclusion, the comments and minor shortcomings identified in this review do not affect my overall positive assessment of the dissertation. Their purpose is to clarify certain issues, refine selected aspects of the work, and encourage scholarly discussion between the Author and the reviewer.

I trust that during the public defence, the Candidate will address these remarks, as well as respond to the additional questions presented below:

1. Could the cross-amplification observed in Figure 2C pose a problem for the detection of food adulteration, particularly in view of the low DNA concentrations of the substituted species?
2. What strategies could be applied in future research to increase the resolution of the method at the species or subspecies level?
3. What health-related and societal consequences may arise from the illegal trade in wild game meat in Nigeria?

Final Recommendation

The doctoral dissertation submitted by MSc Bukola Muibat Adenuga, entitled “Authentication of selected game species in food products based on nuclear markers and qPCR”, constitutes an original and independent solution to a significant research problem and raises no objections of either a formal or substantive nature. The Candidate has addressed a timely and important research issue by developing methods for identifying adulteration in raw materials derived from wild animals and has confirmed that the level of species substitution on the food market remains alarmingly high, thereby further emphasising the applied relevance of the research. In my assessment, the doctoral dissertation of MSc Bukola Muibat Adenuga fully satisfies the requirements set forth for candidates seeking the doctoral degree under the Act of 20 July 2018 – Law on Higher Education and Science. Accordingly, I hereby submit to the Scientific Council of the Discipline of Food Technology and Nutrition at the Poznań University of Life Sciences a motion to accept the dissertation and to admit the Candidate to the public defence.

