OPINION

on the dissertation for the award of the educational and scientific degree "PhD" in the field of higher education: 6. "Agricultural Sciences and Veterinary Medicine", professional field: 6.4. "Veterinary medicine", scientific specialty: "Animal Pathology"

Author of the dissertation: Mag. Majd Ibrahim Abi Haidar, part-time doctoral student at the Department of Internal Diseases, Pathology and Pharmacology at the Faculty of Veterinary Medicine, University of Forestry, Sofia.
Dissertation Title: 'Prevalence and detection of aflatoxin types in dairy cow raw milk raised under different breeding systems, nutrition and season in Bekaa valley'
Member of the Scientific Jury: Prof. Krasimira Ivanova Genova, DVM, PhD, University of Forestry, Sofia, Department of Anatomy, Physiology, and Animal Science at the Faculty of Veterinary Medicine, appointed by Order No. ZPS 646/08.11.2024 of the Rector of the University of Forestry.

1. Relevance of the Problem

Aflatoxins are mycotoxins produced by certain species of Aspergillus, such as A. parasiticus and, less frequently, A. nominus. They contaminate various agricultural products, such as cereals, nuts, and animal feed. It is important to note that aflatoxins, including AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2, can have serious toxic effects on the health of both humans and animals. The highest toxicity is observed in AFB1, which is recognized by the International Agency for Research on Cancer (IARC) as the most hepatocarcinogenic, teratogenic, and mutagenic among the aflatoxins.

Aflatoxin M1 is a hydroxylated metabolite of AFB1 secreted into the milk of mammals that have ingested aflatoxin through contaminated feed. This presents a serious public health risk, as AFM1 is carcinogenic and can enter the human body through milk consumption. With the increasing consumption of dairy products, controlling their quality and safety is of crucial importance.

Changes in climate conditions, such as rising temperatures and increased humidity, favor the growth of molds like Aspergillus. This factor further increases the risk of feed contamination.

The dairy industry in many regions, including Lebanon and the Bekaa Valley, continues to face challenges related to ensuring quality feed and monitoring aflatoxins. The introduction of stricter regulations and standards requires regular studies and research to control aflatoxin levels in milk.

International markets require strict adherence to food safety standards. In the EU and the USA, there are stringent regulations on permissible levels of aflatoxins, and any non-compliance can lead to economic losses and market access restrictions.

Therefore, the study of aflatoxins in milk, especially in regions with active dairy industries like the Bekaa Valley, is a **relevant and important topic, both for public health and the economic sustainability of the sector.**

2. *Degree of Understanding of the Problem and Creative Interpretation of the Literature Review*

The PhD candidate has used 382 literary sources in the research, of which only 14 (3.7%) are from the last 10 years. All of them are cited and integrated into the dissertation in a way that demonstrates their actual usage. Unfortunately, they are not standardized in terms of formatting. The literature used is well selected, but data on the translocational protein ABCG2, which is responsible for the passage of AFM1 into milk, should also be included and interpreted. I cannot accept the inclusion of Wikipedia in the literature references of the dissertation.

From the analysis of the used literature, it can be concluded that **the doctoral candidate has a good understanding of the theoretical context of the issues studied**. This has enabled him to make a summary and apply specific methodological approaches.

3. *Aim, Objectives, and Research Methods in the Dissertation*

The proposed dissertation outlines five main tasks that form the primary approach for

achieving the research objectives. The interrelationship between them is logically structured and aligned with the available methods, ensuring the objectivity of the obtained results. This convincingly demonstrates the doctoral candidate's competence in this key aspect of the research work.

4. *Presentation and Illustration of the Results Achieved by the Doctoral Candidate*

The results of the research conducted in three major dairy regions with different farms fully align with the stated objectives. These results are well-described and systematically organized in the corresponding section, summarized, and presented in 20 tables and 24 figures. The indicators are tracked seasonally, with respect to the influence of climatic conditions (humidity and temperature) on the development of molds and, consequently, the levels of contamination in the feed and milk.

5. *Evaluation of the Results and Literature Used*

The research provides valuable information about the levels of contamination with aflatoxin M1 in raw milk from three regions of the Bekaa Valley, revealing significant correlations between various factors such as farm size, seasonal changes, and climatic conditions. The higher concentration of aflatoxin M1 found in small farms (with fewer than 20 cows) can be explained by less efficient feed quality control systems and lower monitoring levels in these farms.

Seasonal dynamics play a key role in the contamination levels, with the highest values recorded in winter, spring, and summer. This is consistent with the more humid and unfavorable conditions during these seasons, which favor the development of mold in the feed. Winter stands out with a statistically significant difference between the seasons ($p = 0.032$), highlighting the importance of increased monitoring during this period.

The PhD candidate has also paid attention to regional differences in the levels of aflatoxin M1, which should be taken into account when developing strategies to reduce aflatoxin contamination.

It should be emphasized that the dissertation presents sound analyses of the experimental results, which are closely tied to the literature review. This approach demonstrates that

Majd Ibrahim Abi Haidar has mastered contemporary scientific methods. The results obtained and analyzed by him are scientifically grounded

6. *Contributions in the Dissertation*

The careful reading of the dissertation, as well as the publications in which the scientific views and results of Majd Abi Haidar's research work are presented, demonstrate the exceptional interest of the doctoral candidate in the studied topic.

The conducted research has led to results that are of scientific and applied nature and reveal opportunities for future tasks.

I fully agree with the scientific contributions of the dissertation, as outlined by the candidate, which should be presented in the traditional manner – with original or confirmatory characteristics.

7. *Evaluation of the Personal Contribution of the Doctoral Candidate*

My evaluation on this criterion will be based solely on the presentation of the dissertation and publications. The text is written in a way that allows the logical connections between the various stages of the research to be easily traced, clearly demonstrating the validity and personal contribution of Majd Ibrahim Abi Haidar in the presented scientific material. Within my area of expertise, I currently find no grounds or evidence of plagiarism.

8. *Critical Remarks on the Dissertation*

I have no critical comments on the dissertation. Some remarks and suggestions are made in the relevant sections of the report. I would just like to offer a few recommendations. It would be beneficial to direct future efforts towards analyzing the fate of AFB1 after its ingestion by dairy cows, depending on the seasons and the type of feed, as well as addressing issues related to oxidative stress and immunotoxicity

9. *Published Articles and Citations, Quality of the Abstract*

Three publications in refereed journals have been presented on the topic of the dissertation. The doctoral student has also participated in two international scientific conferences.

The presented abstract consists of 36 pages. It is formatted according to the requirements of the relevant regulations. The main chapters of the dissertation are presented, as well as the summarized contributions. The validation of the obtained results is reflected. Unfortunately, the requirement by NAICID for a Bulgarian-language abstract from a doctoral student who is trained in English has caused various issues. Upon reviewing the text, expressions and terms are noticeable that are far from standard usage.

CONCLUSION

The doctoral candidate has presented a completed scientific research, convincingly demonstrating the ability to systematize unresolved issues and scientific theses in the specific scientific field, critically interpret the discussed problems, and successfully conduct independent scientific research. Scientific and applied contributions have been achieved that enrich scientific knowledge and support practice. The dissertation deserves a positive evaluation and meets the requirements for the award of the requested academic degree. All of this provides me with a solid basis to confidently recommend to the esteemed members of the Scientific Jury to confer the **educational and scientific degree of "PhD"** upon the doctoral candidate, **Mag. Majd Ibrahim Abi Haidar**.

05.12.2024 Prepared by:

Sofia (Prof. K. Genova, DVM, PhD)