REVIEW

on a dissertation for obtaining the educational and scientific degree "Doctor" in: Veterinary Medicine, Field of Higher Education 6. Agricultural Sciences and Veterinary Medicine, Professional Field 6.4 Veterinary Medicine, Scientific Specialty: Animal Pathology.

Author of the Dissertation: Majd Abi Haidar

Topic of the Dissertation: *"Prevalence and detection of aflatoxin types in dairy cow raw milk raised under different breeding systems, nutrition, and season in Bekaa Valley."*

Reviewer: Prof. Dr. Rumen Georgiev Binev, Department of Internal Non-Contagious Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora. Appointed as a member of the academic jury by Order № ZPS-646/08.11.2024 of the Rector of LTU.

1. Brief Presentation of the Candidate

Majd Abi Haidar was born on April 25, 1985, in Baskinta, Lebanon. In 2009, he completed a Master's degree (M1) in Life and Earth Sciences, and in 2013, he obtained a Master's degree (M2) in Veterinary Sciences. In 2014, he completed internships in several clinics specializing in ruminants.

Majd Abi Haidar has extensive professional experience as a veterinarian in Lebanon. From 2014 to 2015, he worked as a veterinarian and manager of a farm with 2,500 Alpine and Saanen goats, and from 2015 to 2022, in a farm with 1,500 dairy cows. Since November 2022, he has been working as a hospital veterinarian at the Veterinary Campus in Lyon, France. He has been a lecturer at the Faculty of Agronomy and Veterinary Medicine in Lebanon since 2014. He has participated in four conferences in Spain, Japan, Bulgaria, and France. His professional career

includes numerous internships and training programs, such as an internship in 2008-2009 at the Laboratory of Microbiology and Molecular Genetics in Toulouse, France, and at the Lebanese Institute for Agricultural Research. Between 2009 and 2013, he underwent practical training at the National Veterinary School in Lyon and several clinics in Lebanon, dealing with both productive animals and pets. His skills were further enhanced during the 2013-2014 period through training at three clinics for ruminants in France.

Majd Abi Haidar possesses the necessary academic competence, including knowledge in areas such as physiology, microbiology, and biotechnology, as well as proficiency in various laboratory techniques and analytical skills for interpreting data.

He is fluent in Arabic, French, and English.

2. Structure of the Dissertation

The dissertation is 156 pages long. It contains:

Introduction – 4 pages

Table of Contents – 5 pages

Abbreviations – 4 pages

Introduction – 2 pages

Literature Review – 57 pages

Own Research, divided into:

Objectives and Tasks – 1 page

Materials and Methods – 10 pages

Results – 9 pages

Discussion – 10 pages

Conclusions – 2 pages

Contributions – 1 page

3. Relevance of the Topic

The scientific work aims to determine the levels of aflatoxin M1 in raw cow milk produced in three different dairy regions of the Bekaa Valley, Lebanon. Concurrently, levels of AFB1 in various raw materials used in compound feed production, such as soy, corn, cottonseeds, barley bran, wheat bran, and hay, were measured. Additionally, the impact of geographic region, seasonality, and breeding technology on the spread and development of AFB1 and aflatoxin M1 was established.

Over the last 2–3 decades, there has been widespread occurrence of various mycotoxicoses in animal feed and food products intended for human consumption, including raw milk and dairy products. These mycotoxins have become a global issue, reducing agricultural yields and livestock production. Mycotoxins pose significant risks to both animal and human health, often causing asymptomatic illnesses over long periods while inducing serious damage to parenchymal organs (liver, kidneys, spleen, etc.), the nervous system (central and peripheral), immunity, and are even linked to carcinogenesis in animals and humans.

Therefore, the topic is highly relevant with substantial economic, ecological, and health significance.

4. Literature Review

The literature review is based on 382 sources, all in English. It is divided into 36 sections. The first two sections present general data on the state of the dairy sector in Lebanon and milk and dairy consumption in the country. Sections 3–15 thoroughly describe conditions for contamination, growth, production, toxicity, regulation, and prevention of aflatoxins, focusing on AFB1, and aflatoxin M1.

Information is summarized regarding the physical and chemical properties of aflatoxins, their structure, biological effects, and their presence in various foods and feeds, including raw cow milk and dairy products. The author details maximum permissible concentrations (MPC) of some aflatoxins in feeds and foods and the effects of milk processing methods on aflatoxin M1 content. Methods for reducing aflatoxins in feeds are also discussed.

The sections addressing biological effects of aflatoxins on humans and animals and methods for their analysis stand out with exceptional depth and completeness.

5. Own Research

5.1 Objectives and Tasks

The goal is to determine the level of aflatoxin M1 in raw cow milk from three Bekaa Valley regions and analyze feed samples for AFB1 contamination to identify the main sources of milk contamination.

Five tasks were set:

1. To categorize and select appropriate cow farms in the three dairy regions of the Bekaa Valley.

2. To evaluate the influence of regional differences on aflatoxin M1 contamination in raw milk.

3. To assess the impact of farming type and technology.

4. To evaluate seasonal fluctuations.

5. To analyze AFB1 levels in commonly used feedstuffs.

Listed Tasks and Their Alignment

The listed tasks align with the set objective, and their comprehensive execution forms the foundation for achieving it.

5.2. Materials and Methods of the Study

The research was conducted in three different regions of the Bekaa Valley: Baalbek, Zahle, and West Bekaa. These regions are characterized by distinct farming technologies and varying microclimates.

A total of 352 milk samples were analyzed, distributed across the three regions as follows: 116 from Baalbek, 120 from Zahle, and 116 from West Bekaa. The samples were collected between October 2021 and August 2022.

AFB1 content was measured in a total of 90 feed samples from six components of compound feeds: barley bran, soy, corn, cottonseed, wheat bran, and hay. All feed samples were collected between late June and early July 2022.

Both milk and feed samples were analyzed using the ELISA test. The methodological descriptions are very detailed, clear, and precise.

Statistical analysis of the results was performed using SPSS software version 8.0 for Microsoft Windows (SPSS, Chicago, Illinois).

In summary, the chosen methodological approaches provided the necessary basis for obtaining objective scientific data. The research yielded a significant number of results, some of which represent concrete scientific and practical contributions.

5.3. Results

The study of AFM1 levels in raw milk revealed that only 25.2% of samples in the Baalbek region contained no trace of the metabolite, compared to 55.8% in Zahle and 25% in West Bekaa.

The annual average AFM1 content was measured at 14.31 ng/l in Baalbek, 11.5 ng/l in West Bekaa, and 10.5 ng/l in Zahle.

It was observed that smaller farms (with fewer than 20 cows) exhibited higher AFM1 levels compared to larger farms.

Higher contamination of milk with AFM1 was noted in winter, spring, and summer compared to autumn.

Most results showed that average AFB1 levels exceeded the maximum permissible limits set by the European Union (which are ten times lower than other standards). Additionally, some samples of soy, cottonseed, corn, and hay surpassed the higher limits established in the United States and Lebanon.

Among the raw materials used in compound feeds, corn exhibited the highest level of AFB1, which was attributed to improper storage.

The results are scientifically sound and are thoroughly illustrated with eight tables and six figures, all of which are well-structured and detailed.

Observations:

AFM1 content in milk samples was measured four times, while AFB1 content in feed samples was measured only once. Therefore, the results' significance could have been further enhanced if the metabolic conversion of AFB1 to AFM1 had been studied. This could have been achieved by simultaneously analyzing milk and feed samples across different seasons, regions, levels of milk productivity, and varying farm intensification levels.

6. Discussion

The "Discussion" section of the dissertation offers a detailed and comprehensive analysis of the obtained results. It stands out for its depth of scientific reasoning, which relies not only on the author's findings, but also on numerous studies reflected in the literature.

A primarily comparative and critical approach was used to interpret the observed changes.

7. Conclusions

Based on the author's research, 16 conclusions have been drawn, which are sufficient and well-justified.

8. Contributions

I accept the report on scientific contributions, which include 5 total contributions: 4 original and 1 confirmatory.

9. Recommendations

The conducted research resulted in 6 practical recommendations.

10. Publications Related to the Dissertation

In connection with the dissertation, the author presents 4 scientific papers: 2 of which were presented at international conferences both in the country and abroad, and 2 were published in a journal indexed in NAACID.

The scientific output is sufficient in number and points, meeting the requirements of PZRAS and PPZRAS at LTU – Sofia.

11. Abstract

The submitted abstract accurately reflects the structure and content of the dissertation.

CONCLUSION:

Based on the doctoral candidate's acquired skills in literature analysis, the modern research methods used, the statistical processing of data, the correctly derived experiments, and the conclusions made, I consider the presented dissertation to meet the requirements of ZRARSB and the Regulations of the University of Forestry for its application. This gives me grounds to evaluate it POSITIVELY.

I hereby allow myself to propose to the esteemed Scientific Jury to vote positively and award Majd Abi Haidar the educational and scientific degree "Doctor" in the scientific specialty.

Date: December 5, 2024
Stara Zagora

Reviewer

(Prof. Dr. R. Binev)