ВЕТЕРИНАРНОМЕДИЦИНСКИ ФАКУЛТЕТ

STANDPOINT

of Assoc. Prof. Ivan Valchev Trifonov, PhD
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Regarding: PhD thesis presented by Majd Ibrahim Abi Haidar entitled: „Prevalence and determination of aflatoxins types in raw cow milk, produced in different production systems, nutritional regimens and seasons in the Beqaa Valley", for conferral of the educational and scientific degree PhD, higher education field – 6.0 Aagrarian Sciences and Veterinary Medicine, professional field 6.4. Veterinary Medicine, scientific specialty "Animal Pathology "

1. Structure.

The PhD thesis is written on 156 pages. It contains a preface – 4 pages, table of contents - 5 pages, abbreviations – 4 pages, introduction – 2 pages, literature overview – 57 pages, original studies subdivided into aim and tasks – 1 page, material and methods – 10 pages, results – 9 pages, discussion – 10 pages, conclusions – 2 pages, contributions – 1 page, recommendations for the practice – 2 pages, publications – 1 page, acknowledgements – 3 pages, reference list – 36 pages, appendices – 3 pages.

2. Relevance of the problem.

The topic of the dissertation is **important**, as it concerns aflatoxins, which are natural contaminants of animal feeds and human food. Even in low concentrations, aflatoxins can provoke health problems in animals and humans. They incur significant economic, social, health and environmental problems for humanity. Lebanon is a country in a climatic region, exceptionally

suitable for the development of microscopic aflatoxin-producing fungi, leading to acute and chronic pathologies, including carcinogenicity.

All farm animals are susceptible to poisoning with aflatoxins. When ruminants are fed diets containing aflatoxin B1, this mycotoxin is metabolised in the liver to aflatoxin M1 and passes into the milk. The International Agency for Research on Cancer classifies aflatoxin B1 and aflatoxin M1 as carcinogenic to humans and animals. Aflatoxin M1 is found in the milk of mammals fed diets and food products contaminated with aflatoxins. It is established that in the milk of lactating animals, the transport of aflatoxin B1 as aflatoxin M1 ranges from 0.3% to 6.2% of the total amount of the toxin ingested with feed. Due to its high toxicity and carcinogenicity, aflatoxin M1 is the only mycotoxin for which maximum residue limits in milk have been set. According to the legislation of different countries, the maximum permissible concentrations of aflatoxin M1 in milk range from 20 to 500 nanograms per liter. The main sources of aflatoxins in animal feeds are peanuts, meals, cereals and cottonseed.

At the background of global warming, mycotoxins become one of the most serious challenges in veterinary and human medicine. Moreover, they have an occult and chronic development. Mycotoxicoses occur asymptomatically for a long time, but simultaneously cause irreversible changes in all organs and systems in productive animals, causing huge economic losses. Aflatoxicosis is one of the most common and dangerous mycotoxicoses in livestock husbandry.

3. Literature overview.

The literature overview is based on 382 sources in English. The literature overview is divided into several sections. The first provides general information about aflatoxins regarding their distribution in agricultural products and cow milk, as well as various toxicological characteristics. The second section describes the influence of different milk processing methods on aflatoxin M1 content. The third section describes the biological effects of aflatoxins in humans and different animal species. The fourth section describes various practices for reducing the content of aflatoxins in feed, and the fifth - various methods for detection of aflatoxins. The literature overview contains 35 subsections. The huge number of cited literary sources, mostly published after 2000, show that the PhD student is familiar with the problem on a global scale. All this shows that he has a good awareness of the investigated problem.

Critical remarks

A flaw of the literature review is the lack of a summary of the reviewed literature data. The clearly expressed disproportion when comparing the volume of the individual sections is striking. Among them, the literature review has the largest volume.

4. Aim, tasks, material and methods

The **goal** of the investigated scientific problem is formulated precisely and clearly, focused on 5 tasks.

1. To categorise and select suitable farms for raising cows in the three dairy regions of the Beqaa Valley (Baalbek, Zahlah and West Beqaa).

2. To assess the impact of regional differences on the contamination of raw cow milk with aflatoxin M1 (AFM1).

3. To evaluate the impact of farming type and technology on the contamination of raw cow's milk with aflatoxin M1 (AFM1).

4. To assess the seasonal variations in the contamination of raw cow milk with aflatoxin M1 (AFM1).

5. To evaluate the occurrence and levels of aflatoxin B1 (AFB1) in the most commonly used feeds as source of contamination of raw cow milk with aflatoxin M1 (AFM1).

The **Material and Methods section** is appropriately organised. This section is written on 10 standard typewritten pages. The experimental studies are conducted with a sufficient number of dairy cows in the three geographical regions of Lebanon during the four seasons. A modern enzyme-linked immunosorbent assay (ELISA) was used, with RIDASCREEN® Aflatoxin M1 (R1121) test kit (R-biopharm, Darmstadt, Germany) for the detection of AFB1 and AM1. The methodological approach is described correctly and in detail. All this supports my belief that the results, as well as the conclusions and recommendations for practice made on their basis, are well-founded.

The obtained results are statistically processed using a modern statistical package for social sciences (SPSS version 8.0 for Microsoft Windows; SPSS, Chicago, IL), and the statistical significance was determined through analysis of variance (ANOVA).

Brief notes

It should be noted that despite the large number of dairy cows included in the study, the number of animals from the different Beqaa Valley regions is different.

5. Results.

The "Results" section is very well presented, illustrated with 8 tables and 6 figures. This in turn allows the in-depth analysis of obtained results and their correct interpretation in the subsequent discussion. The style of this section makes clear that the set goal and tasks are fulfilled.

Critical remarks.

The presented results demonstrate that the content of AFB1 in the feed was examined only once, and that of AFM1: four times. It would be more appropriate to determine the amount of AFB1 in the fed diet at each milk sample collection, which in turn would make it possible to determine the correlation coefficient of metabolic conversion of AFB1 into AFM1. On the other hand, this would make possible to determine the season when the largest amount of AFB1 is produced. The literature review describes the clinical signs, laboratory and pathomorphological changes in different types of animals and humans consuming feed and food products contaminated with aflatoxins. As an omission on the part of the author, I have to point out the lack of data on possible changes in the clinical and blood laboratory status, the mortality rate, as well as pathomorphological changes in the target organs for the toxic effect of aflatoxins in the cows included in the performed studies.

6. Discussion.

The "discussion" section contains 11 pages. In this part of the dissertation, a detailed commentary on the obtained results is made. The discussion is done with the necessary competence and depth. The data from the experiments are compared with those obtained by other authors who conducted similar experiments. It should be noted that the author demonstrates the necessary competence and skills to highlight the merits of the conducted scientific research.

7. Conclusions. Contributions. Recommendations.

On the basis on the conducted research studies, 16 conclusions are made, formulated briefly, clearly and accurately, giving a real image of the task set and the derived results.

Five contributions are formulated, of which 4 are original (№1,2,3,4) and one is confirmatory (№5).

Based on the conducted research, six recommendations for practice are made.

In connection with the dissertation, 4 publications are listed, of which 2 reported at international conferences in the country and abroad. Three of the four publications are in editions from the reference list of NACID.

Conclusion

The structure and content of the dissertation „Prevalence and determination of aflatoxins types in raw cow milk, produced in different production systems, nutritional regimens and seasons in the Beqaa Valley", by Dr. Majd Ibrahim Abi Haidar, subject to review, is compatible with the Law on the Development of Academic Staff in the Republic of Bulgaria and the Statute for its implementation. The conducted studies are of theoretical and practical value. The goal and the tasks are successfully realised, original scientific contributions and recommendations for practice have been made. In the dissertation, Dr. Haidar examines a problem of particular importance for human health. The dissertation provides data and information about the variations in the amount of AFM1 in milk of dairy cows depending on climatic conditions and the season. I give a positive evaluation of the reviewed dissertation and propose the honorable members of the Academic Jury to vote positively and confer the PhD educational and scientific degree "Doctor" in the scientific specialty "Animal Pathology" to Dr. Majd Ibrahim Abi Haidar.

03.12.24

Assoc. Prof. Ivan Valchev