

RESPONSE

Of a reviewer for a dissertation of Yanan Wang: «Development and preliminary application of immunochromatography test strips for the detection of double residues of Aflatoxin B1 and Zearalenone», that was submitted for obtaining the scientific degree of Doctor of Philosophy to the one-time academic council at the Sumy National Agrarian University, field of knowledge 21 - "Veterinary Medicine", specialty 211 - "Veterinary Medicine".

1. RELEVANCE OF THE TOPIC OF THE WORK.

Filamentous fungi, particularly *Aspergillus*, *Fusarium*, and *Penicillium*, are capable of producing secondary metabolites known as mycotoxins. Among the many identified toxic metabolites, some of them are potent carcinogens that can provoke acute or chronic intoxications in both humans and animals. The most common mycotoxins in agricultural products are aflatoxins, ochratoxin A, patulin, fumonisins, trichothecenes (deoxynivalenol, toxin T-2 and NT-2) and zearalenone. Some mycotoxicogenic fungi can produce more than one toxin, and some mycotoxins are synthesized by several species of fungi. Compared to other mycotoxins, the safety level of aflatoxins in poultry feed is low; as a result, poultry feed is always at risk of contamination with aflatoxins, which are often found in corn intended for animal feed. When toxigenic isolates of *Aspergillus flavus*, *Aspergillus parasiticus*, or *Aspergillus nomius* grow in poultry feed, they can synthesize various toxic secondary metabolites, including aflatoxin B 1 (AFB 1), aflatoxin B 2 (AFB 2), aflatoxin G 1 (AFG 1), as well as aflatoxin G 2 (AFG 2). As a result, the accumulation of these toxic metabolites in animal tissues can lead to indirect exposure to humans through the consumption of contaminated products such as meat or eggs.

After conducting a thorough analysis of scientific sources on this subject and determining the insufficient level of coverage of issues related to the diagnosis of mycotoxicosis, researcher Yanan Wang took them into account when choosing the topic and areas of research for qualifying scientific work. The qualifying scientific work for obtaining the scientific degree of Doctor of Philosophy of Yanan Wang on theoretical and practical issues is relevant, because it is dedicated to the development and implementation of test strips for the detection of double residues of aflatoxin B1 and zeralenone.

2. THE DEGREE OF JUSTIFIEDNESS OF THE SCIENTIFIC PROVISIONS OF CONCLUSIONS AND RECOMMENDATIONS FORMULATED IN THE WORK.

Scientific provisions, conclusions and recommendations of the dissertation are based on the results of own research. Their plausibility and novelty are substantiated by a large amount of researched biological material using modern microbiological, epizootological, pharmacological, toxicological, hematological with statistical processing of digital indicators.

The materials of the dissertation work are part of comprehensive scientific research of the Department of Veterinary Expertise, Microbiology, Zoohygiene and Safety and Quality of Livestock Products of the Sumy National Agrarian University according to the following thematic plans of research works: "System of monitoring methods of control and veterinary and sanitary measures, regarding the quality and safety of livestock products in diseases of infectious etiology" (state registration No.0114U005551, 20142019) ; "Forecasting the risks of cross border introduction and spread of particularly dangerous animal diseases and the development of scientifically based disinfection systems based on innovative import substitutable highly effective means" (state registration No. 0115U001342, 20182023). And this dissertation is part of the "12th Five-year" National Science and Technology Support Program "Research and Demonstration of Rapid

Detection Technology for Livestock and Poultry Products" (No.2014BAD13B05), the "13th Five Year" National Key Research and Development Plan Program "Food Safety Technology Research and Development" (No.2019YFC1605705) and the Program for Innovative Research Team (in Science and Technology) at the University of Henan Province (20IRTSTHN025).

For the materials of the dissertation, 15 scientific works were published, including: in scientific and professional publications of Ukraine 3, Scopus publication 5, publications in Chinese journal – 2, in conference materials– 4, and 1 methodical recommendation.

3.PURPOSE, RELIABILITY AND NOVELTY OF SCIENTIFIC PROVISIONS, PRACTICAL SIGNIFICANCE, CONCLUSIONS AND RECOMMENDATIONS FORMULATED IN THE WORK.

The purpose of the study Yanan Wang y is to establish a colloidal gold immunochromatographic test strip detection method for AFB1 and ZEN dual residue, so as to provide rapid detection technical support for ensuring the safety of cereal food and feed.

The scientific novelty of the research results is that the For the first time, the immunoreactivity and antibody characteristics of immunogens synthesized by different methods of AFB1 and ZEN are compared and analyzed, and the best immunogens for preparing high specific antibodies and class broadspectrum specific antibodies of AFB1 and ZEN are selected; The animal immunization method for the preparation of highly specific antibodies and the screening method of positive hybridoma cell lines are established, and the highly sensitive and highly specific mAbs against AFB1 and ZEN are prepared; The detection method of AFB1 and ZEN dual residue test strip was established, and its practicability and compliance were verified. The obtained research results are reliable, which is confirmed by digital data, their statistical processing, analysis and discussion.

While appreciating Wang Yanan's dissertation work, I would like to make some comments and get answers to some questions that arose during the work on her dissertation.

In our opinion, tables and graphs should not be used in the first and fourth chapters, but limited to a verbal description of the authors' latest research on this topic.

1. In Figures 3.12 and 3.31, which depict objects under microscopy, in our opinion, it is worth indicating the magnification.

2. I would like to know about the economic efficiency of using the proposed diagnostic strips.

It should be noted that the comments expressed do not affect the positive evaluation of the work, because they do not relate to the essence of the thesis and do not affect the conclusions and proposals for production.

4. APPROVAL OF RESEARCH RESULTS, COMPLETENESS OF SCIENTIFIC PROVISIONS, CONCLUSIONS, RECOMMENDATIONS FORMULATED IN THE WORK.

15 scientific works have been published based on the dissertation materials, including: 3 in scientific and professional publications of Ukraine, 5 in Scopus, 2 in Chinese journals, 4 in conference materials, and 1 methodical recommendation.

The dissertation is presented on 219 pages of computer text, illustrated with 33 tables and 60 figures and consists of an abstract, introduction, review of literature, materials and methods, results of own research, generalization, analysis and discussion of research results, conclusions, proposals, list of used sources, applications. The list of used literature sources includes 295 names.

Scientific provisions, conclusions and recommendations are sufficiently fully set out in published works.

5. CONCERNING THE COMPLIANCE OF THE DISSERTATION WITH THE ESTABLISHED REQUIREMENTS.

The content of Wang Yanan's scientific research definitely corresponds to the specialty 211 - "veterinary medicine". The PhD thesis was performed at an appropriate level and meets the requirements for the design of dissertations and the procedure for awarding the degree of Doctor of Philosophy approved by the resolution of the Cabinet of Ministers of Ukraine dated 12.01.2022 no. 44, which cancels the previous orders of the Ministry of Education and Science of Ukraine dated January 12, 2017 no. 40 and the Ministry of Education and Science of Ukraine dated May 31, 2019 no. 759 with changes and additions. On the introduction of additions to the lists and forms of documents used in the attestation of scientific and pedagogical workers "information on the bioethical examination of dissertation studies for holders of scientific degrees in medical, biological and veterinary sciences". We note that after analyzing the material available to us, no elements of bioethics have been found and no cruelty to animals has been found. All conducted research meets the requirements of the European community.

6. IMPORTANCE FOR SCIENCE AND PRACTICE OF THE RESULTS OBTAINED BY THE AUTHOR OF THE DISSERTATION AND WAYS OF THEIR USE.

In scientific, theoretical and practical aspects, the results presented in the dissertation are relevant, the results of the acquirer's work were focused on the synthesis of ideal immunogens, which solved the problem of low immunogenicity of AFB1 and ZEN; preparation of high-titer, high-sensitivity and high-specificity mAb AFB1 and ZEN mAb, which prevented the occurrence of unstable antibody source and unstable antibody quality. The development of a test strip for the detection of a single AFB1 (or ZEN) residue and a test strip for the detection of double residues of AFB1 and ZEN allowed for quick, simple, repeated detection of mycotoxin residues. Previous application and validation by HPLC-MS/MS of a single residue AFB1 (or ZEN) test strip and a double residue AFB1 and ZEN test strip have demonstrated the practicality and reliability of this method. Thus, effective technical support was obtained for the implementation of rapid detection of AFB1 and ZEN double residues and ensuring food and feed safety. The materials of the dissertation work are used in the teaching of the courses "Veterinary Microbiology", "Veterinary-Sanitary Expertise" for masters of the Faculty of Veterinary Medicine of Sumy NAU and the course "Veterinary Microbiology" for masters of the Henan Institute of Science and Technology (HIST).

Wang Yanan's dissertation work may have further development in scientific research conducted in the direction of monitoring studies of raw materials and food products for the presence of mycotoxins in them.

7. CONCLUSION

The dissertation work of Wang Yanan: «Development and preliminary application of immunochromatography test strips for the detection of double residues of Aflatoxin b1 and Zearalenone», is a completed scientific research work, in terms of relevance, scientific novelty, theoretical and practical significance of the obtained results, it meets the requirements for the design of theses and the Procedure for awarding the degree of Doctor of Philosophy approved by the resolution of the Cabinet of Ministers of Ukraine dated 12.01.2022 No. 44, which cancels the previous orders of the Ministry of Education and Science of Ukraine No. 40 dated January 12, 2017 and No. 759 of the Ministry of Education and Science of Ukraine dated May 31, 2019 with changes and additions, and its author deserves to be awarded the degree of Doctor of Philosophy in specialty 211 - "Veterinary Medicine".

Reviewer,
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R.V. Petrov

