

## RESPONSE

of the official opponent on the PhD thesis Yanan Wang on the topic: «Development and Preliminary Application of Immunochromatography Test Strips for the Detection of Double Residues of Aflatoxin B1 and Zearalenone», submitted for the degree of Doctor of Philosophy in the field of knowledge 21 «Veterinary Medicine» in the specialty 211 «Veterinary medicine»

**Topic relevance.** The rapid development of global agriculture and animal husbandry is accompanied by the contamination of food and feed with mycotoxins, and reports of mycotoxin contamination are increasing worldwide. Mycotoxin contamination can occur in all aspects of crop production, harvesting, storage and processing. The degree of damage is extremely large and the coverage area is wide, which critically threatens the safety of food and feed in the world.

AFB1 and ZEN are the two most common toxins in mycotoxins, which mainly contaminate plant-derived agricultural products, food and feed such as corn, rice, wheat, barley, oats, sorghum, and so on.

AFB1 and ZEN pollution have acute poisoning, chronic poisoning, carcinogenicity, mutagenicity, teratogenicity, neurotoxicity, immunotoxicity, reproductive toxicity and many other toxic effects on human and animal health, especially, AFB1 and ZEN pollution residues often coexist and have synergistic and additive effects, it seriously threatens human health and the development of animal husbandry. Therefore, the establishment of AFB1 and ZEN dual residue detection and the strengthening of AFB1 and ZEN contamination monitoring have become important means to ensure food and feed safety.

The topic of the dissertation is relevant, as the work is devoted to the development and implementation of immunochromatographic diagnosticum using monoclonal antibodies labeled with colloidal gold (GICA; Colloidal Gold Immunochromatographic Assay) in the form of test strips for the simultaneous detection of AFB1 and ZEN mycotoxin residues in order to provide technical support for their rapid detection and guarantee safe consumption of grain food products and fodder.

**Connection of the work with scientific programs, plans, topics.** 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).

**Purpose, reliability and novelty of the scientific provisions, practical significance, conclusions and recommendations formulated in the work.** The aim of the presented PhD thesis 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 author achieved her purpose by studying and development of different immunogen synthesis methods according to the molecular structure characteristics of AFB1 and ZEN. Through the identification of UV, SDS-PAGE and animal immune effect, the best immunogen synthesis methods for preparing AFB1 and ZEN specific antibodies are selected respectively, so as to solve the problems of poor immunogenicity and poor antibody specificity of small molecular compounds of AFB1 and ZEN.

Through the selection of animal immunization methods, cell fusion and positive hybridoma cell line screening technologies, AFB1 mAbs and ZEN mAbs with high sensitivity and high specificity are prepared respectively, and their immunological characteristics are identified, so as to solve the problems of poor quality and unstable source of antibodies.

By way of the preparation and identification of colloidal gold, the preparation and identification of gold-labeled mAbs, the optimization of test strip detection conditions, the assembly and performance measurement of test strip, AFB1 and ZEN dual residue test strip detection methods are established, so as to solve the problems of poor rapidity, poor simplicity, inability to detect on-site, and inability to multiplex detection in the current detection technology.

By means of preliminary practical application and HPLC-MS/MS verification, the practicability and reliability of the AFB1 and ZEN dual residue test strip detection methods are evaluated.

The PhD thesis was completed using sufficient number of laboratory animals and practical material with the involvement of modern methods, namely: mycological, toxicological, material chemical, cytobiological, immunological, pharmacological, diagnostics, clinical and statistical. The animal study of the research was conducted in accordance with the principles of biosafety and bioethics.

The conclusions and proposals for the production of the dissertation work are fully substantiated and correspond to the obtained results of own research.

Scientific novelty of the obtained results is that, for the first time, the immunoreactivity and antibody characteristics of AFB1 and ZEN immunogens synthesized by different methods are compared and analyzed, and the best immunogens for preparing high specific antibodies and class broad-spectrum 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 practical significance of the obtained results is that, for the preparation methods of high-specific antibodies and broad-spectrum specific antibodies to AFB1 and ZEN provide a reference for the preparation of high-quality antibodies to other small molecule haptens compounds.

**The importance for science and national economy of the results obtained by the author of the dissertation, recommendations for their use.** The establishment of AFB1 and ZEN dual residue test strip detection method not only



provides ideas for the research and development of similar detection products, but also improves the methods of controlling the safety of grain food products and feed.

The main provisions of the PhD thesis were included in the Methodological Recommendations «Implementation of modern methods of Aflatoxin B1 and Zearalenone detection» for laboratory, practical classes and independent work for master's students of veterinary department from disciplines «Veterinary Microbiology» and «Veterinary Zoohygiene», specialties: 211 «Veterinary Medicine», 212 «Veterinary Hygiene, Sanitation and Expertise», 53 pp. (approved by Scientific Council of SNUA, protocol No. 5, dated December 29, 2021).

The materials of the dissertation are included in the study guide, work program and lecture course on «Veterinary Microbiology» for the Master's level of educational and qualification training in areas 211 «Veterinary Medicine» and 212 «Veterinary Hygiene, Sanitation and Expertise» in Sumy National Agrarian University and are used in distance education of students on the basis of the «Moodle» platform.

Yanan Wang's dissertation on the topic: «Development and Preliminary Application of Immunochromatography Test Strips for the Detection of Double Residues of Aflatoxin B1 and Zearalenone» is the completed scientific work, completed according to the set goal and objectives. The obtained results are important and reliable, since the work was carried out on a sufficient number of animals with the involvement of modern effective methods of research.

The dissertation is presented on 218 pages of computer text, illustrated with 33 tables and 60 figures and consists of annotation, introduction, review of literature, materials and methods, results of own research, generalization, analysis and discussion of research results, conclusions, proposals, list used sources, applications. The list of used sources of literature includes 295 names, 276 - from far abroad.

**Approbation of research results.** The main provisions and results of the research were reported and received general scientific approval at the annual scientific reports and conferences of faculty and graduate students of Sumy National Agrarian University, Faculty of Veterinary Medicine (2018-2021); Fifth Annual Regional Scientific Symposium One Health Concept, Kyiv (2019). Food Quality and Safety, Health and Nutrition Congress. |Ohrid, Macedonia (2019).

Based on the materials of the dissertation work, 15 scientific works were published, of which 3 articles were published in specialized scientific publications of Ukraine, 2 article was published in a scientific publication of China, 5 articles were published in the Scopus scientific-metric database, in conference materials - 4, and 1 methodical recommendation.

**Personal contribution to solving a scientific problem or solving a specific scientific task.** Under the guidance and help of the supervisors, PhD student had consulted a large amount of literature on the subject of the dissertation, summarized and analyzed the latest progress of the subject research, the main problems solved by the research, and the expected research goals. PhD student had systematically designed research plans and technical routes, selected appropriate technical methods, and carried out relevant experimental research, and performed statistics and analysis



on the test data, discussed and summarized the test results, and drew the correct research results and conclusions of the subject.

The materials of Yanan Wang's dissertation work are practically important and relevant for scientists-mycologists, toxicologists, veterinary workers, pathophysiologists and epizootologists who investigate the problem of mycotoxin contamination of feed and food products.

The results of Yanan Wang's work are aimed at the synthesis of ideal immunogens, which solves the problem of low immunogenicity of AFB 1 and ZEN; preparation of high titer, highly sensitive and highly specific mAb AFB 1 and ZEN mAb, which prevent the occurrence of an unstable source of antibodies and unstable quality of antibodies. The development of test strips for the investigation of a single residue of AFB 1 (or ZEN) and double residues of AFB 1 and ZEN enables quick, simple, repeated detection of mycotoxin residues, which improves and simplifies the control of the safety of feed and food products.

Scientific propositions, conclusions and proposals are formulated accordingly in the dissertation. They are justified and are a logical continuation of the obtained results of experimental research.

**General assessment of the essence of the dissertation, its value and shortcomings in terms of content and design.** Yanan Wang's PhD thesis on the topic: «Development and Preliminary Application of Immunochromatography Test Strips for the Detection of Double Residues of Aflatoxin B1 and Zearalenone» corresponds to specialty 211 – «Veterinary Medicine». The work was performed at the appropriate level and 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 Culture of Ukraine dated January 12, 2017 No. 40 and Ministry of Education and Culture of Ukraine dated May 31, 2019 No. 759 with changes and additions.

Evaluating positively Yanan Wang's PhD thesis on the topic: «Development and Preliminary Application of Immunochromatography Test Strips for the Detection of Double Residues of Aflatoxin B1 and Zearalenone», I would like to make certain shortcomings and get answers to some questions:

1. Not all abbreviations are deciphered in the text and presented in the list of abbreviations (for example, mAbs - monoclonal antibody) or, conversely, the decipherment of some is repeated in the text (Food and Agriculture Organization of the United Nations (FAO)).

2. There are repetitions of phrases in the text of the dissertation (we can recommend, we can recommend; Through the ....).

3. In the subsection «Approbation of dissertation results» the scientific journal is indicated instead of the name of the conference (journal of Sumy National Agrarian University (2018, 2019)).

4. Errors were noted in counting the total number of tables, figures and pages.

5. In the dissertation, the presentation of the results of own research in the work often begins directly with a reference to a figure or table: «The results of .....



were shown in Figure .....»; «The results of ..... were shown in Table.....». It is recommended to start the presentation of the research results with the introductory sentence or actually with the analysis and formulation of the results with a subsequent reference in the text to the corresponding table or figure.

6. What is the priority of the problem of detecting double residues of aflatoxin B1 and zearalenone and the prospects for the use of the presented immunochromatographic test strips in Ukraine, China and the world?

7. Do you have perspective plan to develop analogous diagnostics for other mycotoxins?

The above comments do not reduce the positive assessment of the PhD thesis, because they do not relate the essence of the dissertation work and do not reflect on the conclusions and proposals for production, and the questions posed are clarifying and debatable.

### CONCLUSION

Yanan Wang's PhD thesis on the topic: «Development and Preliminary Application of Immunochromatography Test Strips for the Detection of Double Residues of Aflatoxin B1 and Zearalenone» was performed at the appropriate level, is completed scientific research work, according to 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 Culture of Ukraine dated January 12, 2017 No. 40 and Ministry of Education and Culture of Ukraine dated May 31, 2019 No. 759 with changes and additions, and its author deserves to be awarded the scientific degree of Doctor of Philosophy in the field of knowledge 21 «Veterinary Medicine» in the specialty 211 «Veterinary Medicine».

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Signature of Yevheniia Vashchyk certifies:  
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