

## RESPONSE

of the official opponent to the dissertation work Yanan Wang: "Development and Preliminary Application of test Strips for the Detection of Double residues of AFLATOXIN B1 and ZEARALENON", submitted for obtaining the scientific degree of Doctor of Philosophy to the one-time academic council at Sumy National Agrarian University, field of knowledge 21 - "Veterinary Medicine", specialty 211 - "Veterinary Medicine".

### 1. RELEVANCE OF THE TOPIC OF WORK.

Mycotoxins are widely distributed in the natural environment, mainly contaminating all kinds of grain food and feed, seriously threatening the safety of food and feed in the world, and harming human health and livestock development, causing great concern worldwide. Among the mycotoxins that have been studied in detail to date, there are two of the most significant: AFB1 (aflatoxin B1) and ZEN (zeralenone). The latter pollute the environment and cause great harm.

Dissertation student Yanan Wang, having assessed the insufficient study of the mentioned issues, she considered them when choosing the topic and directions of research for the 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, as it is devoted to the study of the possibility of simultaneous detection of aflatoxin B1 and zeralenone residues in fodder and food products, etc., by means of immunochromatographic diagnostics using monoclonal antibodies labeled with colloidal gold.

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

The high level of scientific results and conclusions given in the dissertation is demonstrated by the objectivity and complex of experimental material confirmed by experimental studies using mycological, toxicological, chemical, cytological, immunological, pharmacological, clinical and statistical research methods.

This dissertation is part of the "12th Five-Year" National Chinese Science and Technology Support Program "Research and Demonstration of Rapid Detection Technology of Various Substances in Livestock and Poultry Products" (No. 20I4BAD13B05), the "13th Five-Year" National Chinese Key scientific and technical program. The research has been carried out in accordance with the tasks of the Food Safety Technology Research and Development Program (No. 2019YFC1605705) and the Program for Innovative Research Group (in Science and Technology) in Henan Provincial University (20IRTSTHN025).

The actual material of the dissertation, conclusions and proposals were approved at the annual scientific reports and conferences of the teaching staff and postgraduate students of the Sumy National Agrarian University, at the meetings of the Scientific Councils of the SNAU and the meetings of the Scientific Council of the Faculty of Veterinary Medicine of the SNAU (2018-2022). Dissertation materials were presented at three state and two international scientific conferences.



The materials of the dissertation work were published in eight scientific works. Four of them were published in the leading journals of the Scopus scientific and metric databases, of which three articles in scientific specialized publications of Ukraine, one article in a scientific publication of China, which is included in the scientific and metric databases.

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

The purpose of Yanan Wang's research is to create an immunochromatographic diagnostic using monoclonal antibodies labeled with colloidal gold (GICA; Colloidal Gold Immunochromatographic Assay) in the form of test strips for the simultaneous detection of mycotoxin residues AFB1 and ZEN, to provide technical support for their rapid detection and guarantee the safe consumption of grain food products and feeds.

The scientific novelty of the research results is that, for the first time, the immunoreactivity of AFB1 and ZEN hapten proteins was compared and analyzed, antigens were synthesized from them using modern methods and chemical compounds, the best antigens (6 types of AFB1 artificial antigen and 5 types of ZEN artificial antigens) were selected to obtain highly specific antibodies to AFB 1 and ZEN. The most effective method of immunization of donor animals to obtain highly specific antibodies and methods of screening obtained with the use of appropriate technologies of hybridoma cell lines were selected, and highly sensitive and highly specific monoclonal antibodies to AFB1 and ZEN were produced.

The results of the author's research can be implemented in the practice of the world's leading countries, and the technology of obtaining diagnostic strips can be taken into account by commercial firms in China and other countries for the development of appropriate test systems. After all, the priority and practical significance of the results are confirmed by the author's publications in the world's leading scientific journals.

In this study, the author used a small dose for immunization (30 pg/cm<sup>3</sup>), a long interval between antigen administrations (4 weeks), administration of antigen to donor animals in the back area (4-6 places), and multiple immunizations (5 times). To control the activity of the received sera, the doctoral candidate used indirect competitive enzyme-linked immunosorbent assay (inELISA) and indirect competitive enzyme-linked immunosorbent assay (icELISA) with monoclonal antibodies to AFB1 and ZEN.

After developing the diagnostics, the author examined 60 natural samples of corn, rice, flour, and fodder. As a result, 39 positive samples were detected, including 22 positive samples from AFB1 and 17 positive samples from ZEN. In addition, the effectiveness of the method for the simultaneous detection of AFB 1 and ZEN toxins developed by the doctoral student using an immunochromatographic test strip with monoclonal antibodies labeled with colloidal gold was confirmed in parallel by the method of liquid chromatography with tandem mass spectrometry (100% coincidence of results) during the detection of these toxins in products food and fodder.

Thus, the author proposed methods of synthesizing ideal antigens, which solved the problem of hapten characteristics of AFB1 and ZEN; preparation of highly sensitive and highly specific monoclonal antibodies to AFB1 and ZEN, which solved the



problems of an unstable source of antibodies and their unstable quality; created test strips for the detection of one of the AFB1 residues (or ZEN separately) and test strips for the detection of both AFB1 and ZEN residues simultaneously, the latter solving the problems of speed, simplicity, multiple detection and spot detection without the use of expensive equipment; control studies of samples of grain products for the presence of AFB1 or ZEN in the variant of test strips with one of the components and with two at the same time showed complete agreement with the results of liquid chromatography with tandem mass spectrometry, showed their practicality and reliability. The introduction of the method of simultaneous detection of AFB1 and ZEN residues with test strips gives impetus to the development of diagnostics for the detection of similar products, in addition, the use of this method guarantees the safety of grain food supplies and feed during consumption of the final product by humans or animals.

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

1. In almost the entire text of the work, the author uses the notation: "... "ml", correct "cm", "1 liter", correct "dm" (in the SI system).

2. About 50% of the abbreviations found in the text are not highlighted in the list of abbreviations (list of symbols). Yes, they are decoded one at a time by the text, but they are not displayed in the list of symbols.

3. Did the author of the study consider the use of specific sera from donor animals (guinea pigs, rabbits) that are larger in volume? After all, the volumes of obtaining sera from white mice, although this involves the use of foreign technology, are minimal.

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 THE STATEMENT OF SCIENTIFIC PROVISIONS, CONCLUSIONS, RECOMMENDATIONS FORMULATED IN THE WORK.

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.

The dissertation is presented on 218 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 titles, 276 from far abroad. The text of the abstract fully corresponds to the text of the dissertation.

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

5. CONCERNING 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 work was performed at the 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 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.

#### 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 made it possible to develop a technology for obtaining immunochromatographic diagnostics using monoclonal antibodies labeled with colloidal gold for the detection of aflatoxin and zeralenone. The author has mastered modern research methods (chemical-toxicological, chromatographic, molecular, etc.) to identify the specified toxins. She took into account the shortcomings of the methods mentioned below and reasonably noted that immunochromatographic methods using test strips are cheaper and less scientifically intensive. The author skillfully implemented all of this in a fairly short period of time.

Based on the analysis of modern specialized literature, the author explained the toxicity and harmful effects of AFB1 and ZEN on animal and human health, analyzed in detail the methods of detection, advantages and disadvantages of indicating double residues of AFB1 and ZEN in food and feed, based on this, she developed a method of immunochromatographic double test strip using colloidal gold to detect AFB1 and ZEN. She used hybridoma methods for obtaining monoclonal antibodies to AFB1 and ZEN, and obtained three cell lines hybridoma of monoclonal antibodies to AFB1 and two ZEN lines, which were used for further work. The immunological characteristics of the obtained hybrid showed the possibility of obtaining significant antibody titers, high specificity and sensitivity of monoclonal antibodies to AFB1 and ZEN, which were subsequently used to establish control methods and develop diagnostics for the detection of AFB1 and ZEN toxins. The author successfully developed immunochromatographic test strips with colloidal gold for detection of residues of AFB1 and ZEN toxins. She developed such strips first for each toxin separately, and showed their high efficiency, and later developed combined strips, with the help of which it is possible to detect both toxins at the same time. The testing of the developed test strips for the study of food products in food and animal feed only confirmed their high efficiency in the detection of mycotoxins.

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

#### 7. CONCLUSION

Yanan Wang's dissertation: "Development and Preliminary Application of test Strips for the Detection of Double residues of AFLATOXIN B1 and ZEARALENON", is a 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 a degree 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 the Ministry of Education and Culture of Ukraine dated May 31, 2019 No. 759 with changes and additions.

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