

RESPONSE

the reviewer for the dissertation Yanan Wang: "Development and preliminary application of immunochromatography test strips for the detection of double residues of Aflatoxin b1 and Zearalenone", 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.

One of the seasonal reasons for a decrease in the resistance and productivity of animals is mycotoxicoses - diseases caused by the impact on the body of the products of life of microscopic fungi. In most cases, their manifestation is a violation of reproductive capacity, disorders of the gastrointestinal tract, metabolic disorders, which subsequently leads to an increase in the cost of treating animals and a decrease in their productivity.

Providing animals with high-quality feed is the main condition for maintaining the health of livestock and obtaining the maximum level of their productivity. But as the temperature rises in the spring period, the quality of fodder may deteriorate. This is primarily due to the active growth of microscopic fungi on nutrient substrates (silage, hay, fodder grain and other feeds). Among the mycotoxins that have been studied in detail so far, there are two of the most significant: AFB1 (aflatoxin B1) and ZEN (zearalenone). The latter pollute the environment and cause great harm. PhD student Yanan Wang, having assessed the insufficient study of the mentioned issues, took them into account 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 Yanan Wang on theoretical and practical issues is relevant, because it is dedicated to the detection of mycotoxins by means of immunochromatographic diagnostics using monoclonal antibodies labeled with colloidal gold and the simultaneous detection of residues of aflatoxin B1 and zearalenone in feed and food products.

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

Yanan Wang's dissertation is based on factual material obtained as a result of methodically correctly conducted research, and the obtained results are processed statistically and their probability is confirmed.

The validity of scientific statements, conclusions and recommendations is confirmed by the high methodological level of the experiments, the logic and sequence of their implementation, the correspondence of research methods to the set goal and task, and the high degree of probability of the results obtained.

The high level of scientific results and conclusions given in the dissertation is demonstrated by the objectivity and totality of experimental material confirmed

by experimental studies using mycological, toxicological, immunological, pharmacological, clinical and statistical research methods.

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, 2014-2019); "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, 2018-2023). 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). 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). journal of Sumy National Agrarian University (2018, 2019). The main provisions of the dissertation were included in the Methodological Recommendations of 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". (approved by the Scientific Council of SNAU, protocol No. 5, dated December 29, 2021).

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

The aim of Yanan Wang's research was 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 cereal food products and feeds.

The scientific novelty of the research results is that for the first time the immunoreactivity of hapten proteins AFB1 and ZEN was analyzed and compared, antigens were synthesized from them using modern methods and chemical compounds, the best antigens (6 types of artificial antigen AFB1 and 5 types of

artificial ZEN antigens) were selected to obtain high specific antibodies to AFB1 and ZEN. The most effective method of immunization of donor animals to obtain highly specific antibodies and methods of screening obtained using 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 PhD student's research can be implemented in the practice of the leading countries of the world, and the technology of obtaining diagnostic strips is recommended to the attention of commercial firms in China and other countries for the development of appropriate test systems. The priority and practical significance of the results are confirmed by the author's publications in the world's leading scientific journals.

In her studies, Yanan Wang used a small dose for immunization (30 $\mu\text{g}/\text{cm}^3$), a long interval between antigen injections (4 weeks), donor animals in several cities of 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 Yanan Wang diagnosticum, 60 natural samples of corn, rice, flour, and fodder were studied. 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 developed method for the simultaneous detection of AFB1 and ZEN toxins using an immunochromatographic test strip with monoclonal antibodies labeled with colloidal gold was confirmed by the traditional method of liquid chromatography with tandem mass spectrometry (100% coincidence of results) during the detection of these toxins in food products and feed.

Yanan Wang proposed methods for synthesizing ideal antigens, which solved the problem of the 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 alone) and test strips for the detection of both AFB1 and ZEN residues simultaneously. The latter solved the problems of speed, simplicity, repeated detection and determination in situ without the use of expensive laboratory equipment; control studies of samples of grain products for the presence of AFB1 or ZEN in the version of test strips with one of the components, as well as with two at the same time, showed complete agreement with the results of liquid chromatography with tandem mass spectrometry, demonstrated their practicality and reliability. The introduction of the method of simultaneous detection of AFB1 and ZEN residues with test strips provides a basis for the development of diagnostics for the purpose of detecting 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.

The obtained research results are reliable, which is confirmed by digital

data, their statistical processing, analysis and discussion.

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. It is desirable to express all data obtained from measurement methods in SI units.

2. Would you not agree that section I "Literature review" would be better expanded and concluded with a well-founded conclusion.

3. In our opinion, it would be better to provide a general scheme of research in the "Materials and methods" section.

4. 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.

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.

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.

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 topic of Yanan Wang's scientific research definitely corresponds to specialty 211 - "Veterinary Medicine". The work was performed at the appropriate level and complies with the Procedure for conducting an experiment on awarding the degree of Doctor of Philosophy, approved by Resolution of the Cabinet of Ministers of Ukraine No. 167 of 03/06/2019. Pursuant to the resolution of the Supreme Administrative Court of Ukraine No. 160 dated March 31, 2005, On making additions to the lists and forms of documents used in the attestation of scientific and pedagogical workers "Information on the bioethical examination of dissertation research 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 the scientific and practical aspects, the results presented in the

dissertation are relevant, they 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 dissertation student mastered modern methods of research (chemical-toxicological, chromatographic, molecular, etc.) to detect the indicated toxins. She proved that immunochromatographic methods using test strips are significantly cheaper and faster.

Based on the analysis of modern specialized literature, Yanan Wang substantiated the toxicity and harmful effects of AFB1 and ZEN on animal and human health, analyzed the detection methods, 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 modern hybrid methods for obtaining monoclonal antibodies to AFB1 and ZEN, and obtained three cell lines of hybrid monoclonal antibodies to AFB1 and two ZEN lines, which were used for further work. The immunological characteristics of the obtained hybrids showed the possibility of forming high titers of antibodies, 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 doctoral student developed immunochromatographic test strips with colloidal gold to detect residues of AFB1 and ZEN toxins. Testing of the developed test strips confirmed their high efficiency in the process of detecting mycotoxins in food products and animal feed.

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

CONCLUSION

The dissertation work of Yanan Wang: "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, according to relevance, scientific novelty, theoretical and practical significance of the obtained results, it meets the requirements of the Procedure for conducting an experiment for awarding the degree of doctor of philosophy approved by the resolution of the Cabinet of Ministers of Ukraine No. 167 dated 06.03.2019, and its author deserves to be awarded the degree of Doctor of Philosophy in specialty 211 - "Veterinary Medicine".

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