

RESPONSE

of reviewer for a dissertation of *Xueqin Zhao*: «Advances in research on mechanism and function of antimicrobial peptides», 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.

Since the discovery of natural antimicrobial peptides capable of broadly targeting multiple pathogens, peptide-based therapeutics have been under the radar of researchers. It is important to focus on synthetic peptides and elucidate their multifaceted mechanisms of action as antiviral, antibacterial, and antifungal agents. Antimicrobial peptides typically affect well-conserved structures such as the phospholipid membrane through pore formation or other constitutive targets such as peptidoglycans in Gram-negative and Gram-positive bacteria and glucan in the cell wall of fungi. In addition, some peptides are particularly active on biofilms, destabilizing microbial communities. They can also act intracellularly, for example, on protein biosynthesis or DNA replication. Their intracellular properties are expanded during viral infection, as peptides can affect several stages of the virus' life cycle, starting from the interaction of the viral receptor with the cell to budding. In addition to mode of action, improvements in manufacturing to increase half-life and performance are also considered, along with advantages and disadvantages for clinical use.

After conducting a thorough analysis of scientific sources on this topic and determining the insufficient level of coverage of issues related to antimicrobial peptide research, researcher Xueqin Zhao took them into account when choosing the topic and directions of research for the qualifying scientific work. Qualifying scientific work for obtaining the scientific degree of Doctor of Philosophy Xueqin Zhao on theoretical and practical issues is relevant, because it is dedicated to the improvement of research methods and the function of antimicrobial peptides.

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 biological material studied using modern microbiological, bacteriological, pharmacological, toxicological, and statistical methods.

The dissertation is a fragment of scientific programs of research work of the National Natural Science Foundation of China (No. 31702259 and 31520103917), Young Talent Lifting Project in Henan Province (2020HYTP041), Key Scientific

Research Projects of Colleges and University in Henan Province (21A230004), Youth Backbone Teacher Project of Colleges and Universities of Henan Province (2020GGJS162), Climbing Project of Henan Institute of Science and Technology (2018JY02) and Program for Innovative Research Teams (in Science and Technology) at the University of Henan Province (20IRTSTHN025). 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 27 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).

According to the results of research, 21 scientific papers were published, including: 4 articles in professional editions of Ukraine, 5 articles in scopuse journals, 2 articles in scientific professional publications of China, 8 theses of scientific reports, 1 – Patent, 1 methodological recommendations.

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

The purpose of the study Xueqin Zhao To investigate mechanism of the antimicrobial peptide mastoparan X in killing Gram negative bacteria in vitro and its anti-inflammatory and barrier repair functions in pneumonia and enteritis diseases in vivo, and to further study the relationship between anti-inflammatory and intestinal microbes of MPX. Finally, to explore the anti-inflammatory and barrier repair functions mechanism of MPX in the intestinal epithelial cells, laying a foundation for reducing the use of antibiotics in livestock and poultry breeding, it will help provide certain theoretical and practical value for future applications in livestock and poultry.

The results of this study lay the foundation for the development and use of clinical antibacterial drugs against *A. pleuropneumoniae* and *E. coli*. This also lays the foundation for the selection of anti-inflammatory drugs and drugs to fight intestinal microflora. In addition, it provides theoretical support for decreasing clinical antibiotic resistance against *A. pleuropneumoniae* and *E. coli* infection.

The main provisions of the PhD thesis were included in the Methodological recommendations "Prevention of antibiotic resistance through the use of antimicrobial peptides", approved by the Scientific Council of the SNAU (Protocol No. 5 dated 12.29.2021).

The obtained research results are reliable, which is confirmed by digital data, their statistical processing, analysis and discussion.

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

1. In our opinion, it is not appropriate to use drawings in the first chapter of the dissertation.

2. Why did you choose the causative agents *A. pleuropneumoniae* and *E. coli* to achieve your goal?

3. In our opinion, it is appropriate to include digital material in the "Conclusions" section.

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.

Based on the materials of the dissertation, 21 scientific works were published, including: 4 articles in specialized publications of Ukraine, 5 articles in scopus magazines, 2 articles in scientific specialized publications of China, 8 theses of scientific reports, 1 – patent, 1 methodological recommendations.

The dissertation is presented on 180 pages of computer text. The work is illustrated with 6 tables, 39 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, appendices. The list of used literature sources includes 230 titles.

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 Xueqin Zhao'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 researcher's work were focused on the well-founded mechanism of bactericidal and anti-inflammatory action, barrier protection, and the relationship between the anti-inflammatory action and the intestinal flora of the antimicrobial peptide. for the further development of drugs with the aim of preventing inflammation, maintaining the balance of intestinal flora and improving productivity.

The study investigated the mechanism of the antimicrobial peptide mastoparan X, its ability to kill gram-negative bacteria in vitro, and its anti-inflammatory and barrier repair functions in pneumonic and enteric diseases in vivo, as well as to further study the relationship between anti-inflammatory and intestinal microorganisms. The anti-inflammatory and barrier repair mechanism of MPX in intestinal epithelial cells was also investigated, laying the foundation for reducing the use of antibiotics in livestock and poultry production, this will help to provide some theoretical and practical value for the future application of MPX in animal and poultry disease prevention.

Xueqin Zhao's dissertation work may have further development in scientific research, for further development of drugs to prevent inflammation, maintain the balance of intestinal flora and improve productivity.

7. CONCLUSION

Xueqin Zhao's dissertation work: " Advances in research on mechanism and function of antimicrobial peptides" 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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