

## RESPONSE

of the official opponent for the dissertation work of **Zhao Xueqin** on the topic: "**Advances in research on mechanism and function of antimicrobial peptides**" applied for 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".

### **Relevance of the work**

The bactericidal mechanism, barrier protection, anti-inflammatory effect and the relationship between the anti-inflammatory effect and the composition of the intestinal microflora when using an antimicrobial peptide are substantiated on the basis of the conducted research in the dissertation. Despite the fact that antibiotics have antibacterial and anti-inflammatory effects, they can damage the function of the intestinal barrier and negatively change the composition of the intestinal microflora. The problems caused by the long-term uncontrolled usage of antibiotics become to be more and more serious. Development of the effective and safe substitutes for them is an inevitable process in the development of antibacterial therapy.

Antimicrobial peptides are one of the alternatives to conventional antibiotics that are currently being actively researched and attracting significant attention from the scientific community. Antimicrobial peptides are a promising alternative to antibiotics due to their effective broad-spectrum antimicrobial action, lack of resistance of microorganisms to them, and other types of biological activity.

Antimicrobial peptides could be received from a wide range of sources found in most organisms, and can be isolated from insects, invertebrates, mammals and even humans. Antimicrobial peptides are low molecular weight peptides with short amino acid sequences (no more than 100 amino acid residues).

Zhao Xueqin's dissertation on the topic: "Advances in research on mechanism and function of antimicrobial peptides" is aimed at a detailed analysis, development and justification of the mechanism of action of antimicrobial peptides, their antibacterial effect, formation of anti-inflammatory and barrier functions.

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

The dissertation was completed in accordance with the plans of research works: National Natural Science Foundation of China (Nos. 31702259 and 31520103917), Henan Province Young Talent Development Project (2020HYTP041), Henan Province Colleges and Universities Key Research Projects (21A230004), Henan Province Colleges and Universities Youth Teachers Project (2020GGJS162), Project promotion of Henan Institute of Science and Technology (2018JY02) and Program for Innovative Research Groups (in Science and Technology) in Henan Provincial University (201RTSTHN025). The materials of the dissertation are also part of comprehensive scientific research of the Department of veterinary expertise, microbiology, zoohygiene and safety and quality of animal husbandry products of the Sumy National Agrarian University: "System of monitoring control methods and veterinary and sanitary measures regarding the quality and safety of animal husbandry products in diseases of infectious ethiology" (state no. registration number 01 14U005551,2014-2019).

**Purpose, reliability and novelty of the scientific provisions, practical significance, conclusions and recommendations formulated in the work.**

The aim of the dissertation is to investigate the mechanism of action of the antimicrobial peptide mastoparan X (MPX) on gram-negative bacteria *in vitro* and its anti-inflammatory and barrier recovery function in pneumonia and enteritis diseases *in vivo*, as well as to further study the relationship between the anti-inflammatory effect of MPX and intestinal microflora. Finally, studying the mechanism of anti-inflammatory and barrier repair functions under the influence of MPX in intestinal epithelial cells, laying the foundation for reducing the use of antibiotics in livestock and poultry production, will help to provide some theoretical and practical value for future applications in livestock production, and birds

The dissertation student achieved her goal by studying the mechanism of the antimicrobial action of MPX *in vitro* on *A. pleuropneumoniae* and the anti-inflammatory effect of MPX against pneumonia caused by *A. pleuropneumoniae* infection *in vivo*. The mechanism of the bactericidal effect of MPX on *E. coli in vitro* was also studied in this work. The effects of MPX against *E. coli* infection-induced intestinal inflammation and intestinal barrier protection *in vivo* were investigated. The mechanism of the anti-inflammatory and protective barrier function of MPX at the cellular level has been determined. In addition, the relationship between the anti-inflammatory effect and the intestinal flora of MPX was further analyzed in mice.

The work was performed on a sufficient number of laboratory animals and practical material with the involvement of modern methods, namely: microbiological (PCR studies), pathomorphological, toxicological (acute and chronic toxicity), serological, physicochemical (pharmacopenetics, pharmacodynamics), statistical (processing of results of the study). 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.

The scientific novelty of Zhao Xueqin's dissertation is that the mechanism of the antibacterial effect of antimicrobial peptide MPX on *A. pleuropneumoniae* and *E. coli* was studied for the first time, the study of the anti-inflammatory and protective barrier defense mechanism at the cellular level.

**The importance for science and national economy of the results obtained by the author of the dissertation, recommendations for their use.**

The results of the work lay the basis for the development and use of clinical antibacterial drugs against *A. pleuropneumoniae* and *E. coli*. Also obtained can be used for the selection of anti-inflammatory drugs and drugs to fight intestinal microflora. In addition, the obtained material provides theoretical insight for reducing clinical antibiotic resistance against *A. pleuropneumoniae* and *E. coli* infection. The main provisions of the candidate's thesis were included in the "Methodological recommendations on the mechanism and function of antimicrobial peptides, approved by the Scientific Council of the SNAU (protocol No. 9 dated March 30, 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 21.1 "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.

**Zhao Xueqin's** dissertation 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 main content of the dissertation is presented on 180 pages of computer text and illustrated with 6 tables and 39 figures. The work consists of an introduction, a literature review, research materials and methods, the results of own research, analysis and generalization of research results, conclusions, proposals for production, a list of used literature sources, which consists of 230 names and 5 appendices.

**Approbation of research results, completeness of the statement of scientific provisions, conclusions, recommendations formulated in the work.**

The main provisions of the dissertation were presented, discussed and approved at the meeting of: annual scientific and practical conference of teachers, graduate students and students of Sumy National Agrarian University, Sumy, 2018-2021; BTRP Ukraine Regional One Health Research Symposium (May 20- 24, 2019, Kyiv, Ukraine); Chinese Society of Veterinary Microbiology 2021 Academic Forum (June 19-21, 2021, Zhengzhou, China); One Health International Student Conference (November 24 - 27, 2021, Bucharest, Romania).

The main results of the research on the topic of the dissertation were presented in 21 scientific works, of which 4 were articles in scientific specialized professional publications of Ukraine, 5 were in periodical scientific publications, included in the scientific and metric databases of Scopus, 2 - articles in Chinese scientific publications, 8 - abstracts of scientific reports, 1 - patent, and one - methodical recommendations.

**Personal contribution to solving a scientific problem or solving a specific scientific task.**

The author personally participated in the implementation of the scientific programs that formed the basis of the PhD thesis; she developed schemes and methods of conducting experiments in the laboratory. Setting tasks, discussing results, forming conclusions were carried out together with the manager. The graduate student analyzed the literature and conducted a patent search on the topic of the dissertation; experimental studies were carried out using modern methods with co-authors of scientific papers. The winner has written a dissertation and published articles that outline the main material of the candidate's dissertation. The author thanks Dr. Herds and the laboratory staff for their help.

The materials of **Zhao Xueqin's** dissertation work are practically important and relevant for microbiological scientists, veterinary workers, pathophysiologists and epizootologists who study this problem.

Scientific propositions, conclusions and proposals, logically formulated in the dissertation. They are justified and followed on obtained results of the experimental study.

The applicant personally prepared the dissertation work, conducted a search and

analysis of scientific sources on the topic of the dissertation, prepared a literature review, selected material, carried out experimental research using the selected methods, statistical processing and analysis of the obtained data.

**General assessment of the essence of the dissertation, its value and shortcomings in terms of content and design.**

**Zhao Xueqin's** dissertation on the topic: "Advances in research on mechanism and function of antimicrobial peptides" corresponds to 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.

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

1. In the text of the dissertation, there are unfortunate expressions such as "According to the manual.." in my opinion, it should be: "According to the methodology in the manual.."

2. "Fluorescent microscopy showed..." instead, it is better to say "the results were obtained using fluorescence microscopy..."

3. Also, references to tables are not always present in the dissertation research. In the process of reviewing the dissertation, some questions arose:

1. Please tell me why you focused on the pathogens *A. pleuropneumoniae* and *E. coli* in your work?

2. Do you think MPX will work against non-museum isolates of *A. pleuropneumoniae* and *E. coli*?

3. Please tell me whether in your study you determined the effect of MPX on the composition of the intestinal microbiome?

4. In your work, you indicate that MPX changed the composition of the intestinal microflora of mice infected with *E. coli*, increasing the number of beneficial bacteria and decreasing the number of harmful bacteria. Let me know, do you plan to conduct the further research this effect on pigs or other productive animals?

However, despite some minor shortcomings, the dissertation made the positive impression. It should be noted that the comments expressed do not affect the positive evaluation of the presented work, because they do not relate to the essence of the thesis and do not affect the conclusions and proposals for production.

**Conclusion**

The PhD student has performed well-founded research, presented in a consistent form, and made reasoned conclusions and practical suggestions arising from the results obtained.

We believe that **Zhao Xueqin's** dissertation on the topic: "Advances in research on mechanism and function of antimicrobial peptides" was prepared in

accordance with the order of the Ministry of Education and Science of Ukraine dated January 12, 2017 No. 40 "On approval of requirements for the preparation of a dissertation" and the Ministry of Education and Science of Ukraine dated May 31, 2019 No 759 with changes and additions, is the completed scientific research work, which in terms of relevance of the chosen topic, scientific novelty, theoretical and practical significance of the received results, level and scope of the performed research, fully meets the requirements stipulated by the Procedure for awarding the degree of Doctor of Philosophy approved by the resolution of the Cabinet of the Ministers of Ukraine dated 12.01.2022 No. 44, and its author deserves to be awarded the educational and scientific degree of Doctor of Philosophy in the field of knowledge 21 "Veterinary Medicine" in the specialty 21 1 "Veterinary Medicine".

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