RESPONSE

official reviewer for the dissertation work of Ping Xu: "Anti-inflammatory effect and mechanism of Chlorogenic acid extract from Taraxacum officinale on LTA-induced mastitis in dairy cows", which was submitted for the scientific degree of Doctor of Philosophy to a one-time medical examination at the Sumy National Agrarian University, branch of knowledge 21 – "Veterinary medicine", specialty 211 – "Veterinary medicine".

1. Relevance of the topic of the work.

Mastitis is one of the most prevalent and costly diseases of dairy cows worldwide. Treatment of 1 cow costs from 1 to 2 thousand dollars a year. The causes and management of bovine mastitis are complex, but various measures can be implemented to optimize udder health and productivity in dairy herds.

Economic losses stem from reduced milk production and decreased milk quality. Farmers must discard milk from cows with clinical cases of mastitis and from cows undergoing antibiotic treatment according to withdrawal periods in order to provide time for antibiotics to clear the cow's body.

Mastitis also alters the composition and properties of milk, resulting in reduced cheese yields and reduction of shelf life of manufactured dairy products. Treatment costs and veterinary costs rise, as do labor costs, and milking parlor efficiency can decrease due to increased time spent attending to mastitic animals.

In addition to economic losses, animal welfare is a concern as studies have shown that mastitis can be painful and cause discomfort to cows. Thus, cows diagnosed with clinical mastitis, or those with persistent subclinical mastitis have a greater risk of being culled.

Indeed, udder health issues are frequently cited as one of the top three reasons for culling of dairy cows. Low milk production, potentially associated with mastitis, is another leading cause of culling in dairy herds.

2. Degree of justifiedness of the scientific provisions of the conclusions and recommendations formulated in the work.

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); Twentieth Chinese National Conference on Animal Genetics & Breeding, Guangzhou (2019); The 2021 Academic Forum of the Veterinary Microbiology Committee of the Chinese Society of Microbiology, Zhengzhou (2021); The 7th International Symposium on Dairy Cow Nutrition and Milk Quality, Beijing (2021).

Based on the materials of the dissertation, 20 scientific works were published, including 4– articles in scientific professional poublications of Ukraine, 3 – articles in scopuse journals, 3- articles in scientific professional poublications of China, 9 – Theses of scientific reports, 1 – methodological recommendations.

The dissertation work is carried out in accordance with the programs of research work of Sumy National Agrarian University "Optimization of a complex of antiepizootic measures in the farms of the North-East region of Ukraine" (N_{2} DR 0114U001561) and "To develop a system of control of epizootic well-being for infectious diseases, for the diagnosis of infectious diseases and assessing the safety of livestock and poultry products in Northeastern Ukraine (No. DR 0114U000261). The work was carried out for the period from 2018 to 2022 at the departments of veterinary examination, microbiology, zooghygiene and safety and quality of animals products, the department of epizootology and parazitology and the department of virology, pathanatomy and bird diseases of Sumy National Agrarian University. In addition, veterinary studies were conducted at Henan, China.

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 bacteriological, pharmacological, toxicological, immunological and statistical methods.

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

The purpose of this study is to isolate, extract, detect the content and antibacterial mechanism of chlorogenic acid extract in Taraxacum officinale, and evaluate the anti-inflammatory effect and mechanism of chlorogenic acid extract from Taraxacum officinale in the LTA-induced bovine mammary epithelial cell in vitro inflammation model.

For the first time, chlorogenic acid (CGA) in dandelion (Taraxacum officinale) was conducted extraction and detection. A high performance liquid chromatography method was established for simultaneous detection of five phenolic acids in dandelion , and the method was efficient and stable. Antibiofilm activity against Staphylococcus aureus of Taraxacum Officinale phenolic extract was determined. In vitro culture and evaluation of bovine mammary epithelial cells from Ukraine dairy cows was conducted. Inflammatory model of bovine mammary epithelial cells (BMEC) induced by LTA was established. At last, the effect of chlorogenic acid extract on inflammatory response induced by LTA in BMEC was studied.

This study determined the contents of chlorogenic acid, caffeic acid, rutin, luteolin and ferulic acid in the phenolic extract of the whole dandelion plant by HPLC, which were 1.34 mg/g, 0.21 mg/g, 0.19 mg/g, 1.08 mg/g, and 0.22 mg/g, respectively.

The results of the author's research can be implemented in the practice of most countries, and the technologies proposed in the research can be used for the development of modern medicinal products using Taraxacum officinale.

Appreciating Ping Xu'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 addition to studying the clinical forms of mastitis in cows, it would be desirable to pay attention to the subclinical form.

2. When studying the therapeutic effectiveness, it would be better to expand the spectrum of mastitis pathogens, including not only S. aureus, but also other bacteria and possibly mycoplasma.

3. When determining the therapeutic effect of Taraxacum Officinale in mastitis in cows, it would be desirable to compare it with conventional treatment methods.

4. Approval of research results, completeness of the statement of scientific provisions, conclusions, recommendations formulated in the work.

The dissertation is presented on 146 pages of computer text, illustrated with 15 tables and 38 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 182 names. 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 scientific research carried out by Ping Xu 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 CMU 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.

The results presented in the dissertation made it possible to develop methods of antibiotic-free treatment of inflammation of the mammary gland in cows in scientific, theoretical and practical aspects. Their author has mastered modern research methods, which include bacteriological, pharmacological, toxicological, immunological and statistical methods.

This research takes chlorogenic acid as the research object, which is a natural ingredient in the Chinese herbal medicine Taraxacum officinale. Firstly, the optimal extraction conditions of chlorogenic acid in dandelion were determined by orthogonal experiment. A high performance liquid chromatography (HPLC) method for the detection of chlorogenic acid in dandelion was established. Secondly, detection of the antibacterial mechanism of phenolic acid extract (include chlorogenic acid) against Staphylococcus aureus. Lastly, the mammary epithelial cells were isolated and cultured from milk, and obtained pure primary cells; BMEC inflammatory model was established by Lipoteichoic acid (LTA); CCK-8, ELISA, qRT-PCR and WB were used to analyze the protective and anti-inflammatory effect of chlorogenic acid on LTA-induced inflammatory lesions. In order to provide a reference for the Chinese herbs treatment of dairy cow mastitis, it provides scientific basis for the research and development of Chinese herbs and related basic research.

In the study of detection and extraction process of chlorogenic acid extract from Taraxacum officinale. The extraction technology of chlorogenic acid was researched from multiple factors, the best parameters of the technology were obtained that the ultrasonic temperature was 80 0C, the solid-liquid ratio was 1 : 30, the solvent concentration was 50%, and the ultrasonic time was 40 min, then the extraction rate can reach 1.921%. The HPLC detection method of chlorogenic acid from Taraxacum officinale was 5% methanol elution condition $0 \sim 5 \text{ min}$, 5 - 15% methanol elution condition $5 \sim 15 \text{ min}$, 15 - 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $15 \sim 20 \text{ min}$, 5% methanol elution condition $20 \sim 25 \text{ min}$, and the buffer salt was 1% phosphoric acid aqueous solution, and the wavelength was 350 mm.

In the process of the antibacterial experiment, it was found that phenolic extract from T. Officinale has an inhibitory effect on S. aureus, and the mechanism of action was to destroy the integrity of its cell walls and cell membranes. The minimum inhibitory concentration (MIC) of dandelion phenolic extract exhibited against S. aureus was

12.5 mg/mL. The antibacterial kinetic curve analysis showed that the inhibitory effect of dandelion phenolic extract on S. aureus was mainly in the exponential growth phase. After the action of dandelion phenolic extract, the growth of S. aureus was obviously inhibited entering into the decay phase early. Furthermore, after the action of dandelion, the extracellular AKP contents of S. aureus, the electrical conductivity and the extracellular protein contents were all increased. The phenolic extract also affects the normal reproduction of S. aureus.

7. Conclusion.

The dissertation work of Ping Xu: "Anti-inflammatory effect and mechanism of Chlorogenic acid extract from Taraxacum officinale on LTA-induced mastitis in dairy cows", is a completed research work, in terms of relevance, scientific novelty, theoretical and practical significance of the obtained results, it meets the requirements regarding the design of theses and the Procedure for awarding the degree of Doctor of Philosophy approved by the CMU Resolution No. 44 of 12.01.2022, which cancels the previous orders of the Ministry of Education and Culture of Ukraine No. 40 of January 12, 2017 and No. 759 of the Ministry of Education and Culture of Ukraine dated May 31, 2019 with changes and additions.

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