

OPINION

On a dissertation for obtaining the educational and scientific degree "Doctor" in the scientific specialty "Obstetrics and gynecology of animals and diseases of newborn animals", field of higher education 6.0 Agricultural sciences and veterinary medicine, professional direction 6.4. Veterinary Medicine.

Author of the dissertation: Assistant professor Alexander Alexandrov Stoimenov, University of Forestry, Sofia

Dissertation topic: "Studies on the prevalence, etiology and natural defense mechanisms of the mammary gland in sheep with subclinical mastitis "

Opinion prepared by: prof. Teodora Petrova Popova, DVM, DSc, University of Forestry, city of Sofia, field of higher education "Agrarian sciences and veterinary medicine", professional direction 6.4. "Veterinary medicine", scientific specialty "Epizootology, infectious diseases and prevention of contagious diseases in animals", appointed as member of the Scientific jury by Order No. ZPS 640/01.12.2022 of the Rector of the University of Forestry in Sofia,

• Brief introduction of the candidate.

Alexander Alexandrov Stoimenov graduated in 2018 from the University of Forestry (UF) in Sofia with a Master's degree, professional qualification "Veterinary Doctor", specialty "Veterinary Medicine". He gained practical experience in his specialty by completing a number of internships and trainings. During the period 01.02.2017 - 30.08.2018, he worked as an intern veterinarian at "Animal Rescue" in the city of Sofia, specializing in the field of diagnostics and treatment of companionhip animals. Immediately after that, he started working in his specialty as a veterinarian at the Veterinary Center "St. Antim" in the city of Sofia, where he completed a course on practical and theoretical training of students. Immediately after that, after passing a competition, he entered the University of Forestry in Sofia as an assistant professor. A. Stoimenov participated in the Workshop "Parasitic and Infectious Practices - KRKA 2018", as well as in the International scientific conference "Tradition and modernity in veterinary medicine" of the FVM, UF in 2019 and 2021. He speaks English and has computer and digital skills of high level. He has also acquired knowledge and experience in working with highly specialized medical equipment and interpreting the results, as well as with laboratory equipment for scientific research.

Alexander Stoimenov is enrolled in doctoral studies in an independent form of study by Order of the Rector of UF No. ZSD 154/28.06.2021, with scientific consultant Assoc. Prof. Dr. Kalin Yordanov Hristov. He completed the individual study plan of the doctorate, successfully passed three exams in the specialty and by Order of the Rector of UF No. ZSD - 499 /03.11.2022 he was awarded the right to defend his dissertation. Assistant Prof. Al. Stoimenov has fulfilled the minimum national requirements for the acquisition of the ESD "Doctor", which is also certified by the Opinion of the Head of the "Academic Staff" sector at UF, Sofia (Exit No. 901/08.11.2022).

• General characteristics and structure of the dissertation.

The dissertation is written on 175 standard pages. It includes the necessary sections according to generally accepted requirements: table of contents (1 page), list of abbreviations used in the text (2 pages), introduction (3 pages), literature review (52 pages), purpose and tasks (1 page), materials and methods (16 pages), results (34 pages), discussion (25 pages), conclusions (1.5 pages), contributions (1 page), recommendations for practice (0.5 pages), list of scientific publications related to the dissertation work (0.5 p.), acknowledgments (1 p.) and bibliography (34 p.). This description of the

sections included shows that **the dissertation is structured appropriately and correctly, with an adequate ratio between the sections.**

- **Relevance of the problem.**

Sheep farming is a fundamental branch of animal husbandry that has been practiced since ancient times. Today it is being developed in many countries around the world. In our country at this stage, it is significantly limited, but it has its role in the economy of our country. In terms of chemical composition, sheep's milk is significantly superior to the milk of other farm animals and is very well absorbed by the body. It contains larger amounts of fats, proteins, macro- and micro-elements, vitamins and other valuable ingredients and is extremely useful for health as a nutritional as well as a therapeutic product, especially in case of a deficiency of calcium, vitamin B12 and folic acid. However, one of the main problems in sheep farming are inflammations of the mammary gland (mastitis). They cause significant economic losses. The main reason for their development is the lack of knowledge and non-observance of the basic zoohygiene requirements for raising sheep and especially incorrect application of machine milking. Unlike clinically manifested forms of inflammation of the mammary gland, subclinical mastitis is difficult to diagnose. As a result, there is a decrease in milk production and the quality indicators of milk, an increased risk of spreading the disease in healthy animals and sometimes irreversible changes in the parenchyma of the gland. This requires the study of this problem in our country and the search for modern diagnostic methods for early detection of changes in milk, the mammary gland and its protective mechanisms. In this aspect, **the topic of the presented dissertation is relevant.** It is related to a study of the prevalence, etiology and diagnosis of subclinical mastitis in dairy sheep in our country, as well as changes in the cytological composition and physico-chemical parameters of milk and basic protective mechanisms of the mammary gland, with a view to the prevention of these diseases and their early diagnosis and therapy.

- **Degree of knowledge of the state of the problem and creative interpretation of the literature review.**

The introduction of the dissertation is thorough and clearly presents the problem under consideration.

The literature review examines the structure and physiology of the mammary gland in sheep. The physicochemical composition of sheep's milk is described, as well as the factors that influence it. The basic anatomical, cellular and molecular protective factors and mechanisms of the gland are very well presented. Information on the classification of mastitis in sheep is given and its distribution worldwide is detailed. Their etiology is reviewed, as well as the resistance of the causative agents to antimicrobial means, and up-to-date data are presented. The predisposing factors for their development are described, as well as the methods of diagnosis of these diseases. The treatment and prevention of mastitis in sheep are presented in detail. The review ends with a brief summary of the literature information, giving direction to determine the purpose and tasks of the dissertation work.

Some typewritten, spelling, and technical errors are found in the text of the overview, as well as in other sections of the dissertation, e.g. an abbreviation of a term is given in parentheses 2-3 times after the initial indication of the abbreviation, also in places it is tolerated to write species epithets of bacteria with a capital letter. Also, once the names of microorganisms are spelled out in full, they should be abbreviated later in the text. This rule is not followed in many places, e.g. in the literature review (pp. 30 – 33) also in the discussion, etc. There is repetition of text, e.g. the first paragraph on page 38 coincides maximally with the second paragraph on page 14.

The literature review is structured appropriately and prepared competently. It is fascinatingly written and reads with interest and satisfaction from the doctoral student's excellent awareness and theoretical training, as well as from his skillful handling of specialized terminology and scientific data. It clearly shows the high awareness of the author on the topic of the dissertation work.

- **Purpose, tasks, hypotheses and research methods. Correspondence of the chosen research methodology with the set goal and tasks of the dissertation.**

The purpose of the dissertation is clearly and correctly formulated. It envisages studies on the spread of subclinical mastitis in sheep for milk production in our country, on the microorganisms involved in the etiology of the disease, their sensitivity to antimicrobial agents, on changes in the cytological composition and physicochemical indicators of milk, on some of the protective mechanisms of the milk gland, as well as the possibilities of application of non-contact thermography for rapid diagnosis of subclinical mastitis in sheep. **The tasks are five in number, correctly defined and clearly formulated. They are appropriate and sufficient to achieve the purpose of the dissertation work.**

In the "Materials and methods" section, the animals used, the technology of their breeding and milking, the studied farms, the number and method of taking the samples for research are very well presented. The experimental settings of the conducted epizootological surveys on the spread of subclinical mastitis in sheep farms from different regions of the country are described, as well as to determine the microbiological characteristics of the causative agents and their antibiotic sensitivity, the changes in the cytological composition and the physicochemical parameters of milk in lactating sheep with subclinical mastitis, of humoral and cellular defense mechanisms. State-of-the-art materials and consumables from reputable manufacturers have been used, which is an additional guarantee of obtaining accurate results.

In section 4.2. it would be appropriate to indicate the methods used to determine the indicators of fat content, protein, dry matter, dry defatted residue, freezing point. It is necessary to take into account that ml should not be followed by a period, also a uniform designation of this indicator should be adopted throughout the development, and not "mL" or "ml", as it is now in the different sections of the dissertation. For the concentrations of the used suspensions, the superscript of the exponents has been omitted.

The applied method of non-contact thermography for the diagnosis of inflammatory processes of the mammary gland is clearly presented. The method of obtaining the milk and blood samples for the laboratory tests is described. Applied research methods are also well presented, including clinical methods for determining the general health status of sheep and the state of the mammary gland, laboratory methods for milk research (cytological, physicochemical, microbiological, hematological, biochemical and immunological), thermographic tests, as well as the statistical processing of the results. This shows the doctoral student's good awareness of the applied methodology and his significant participation in the research. The number of experimental and control samples examined was sufficient to obtain statistically significant results. **The selected research methods are appropriate, contemporary, and fully correspond to the set goal and tasks of the dissertation work.**

- **Visualization and presentation of the obtained results.**

The results of the conducted research **fully correspond to the set tasks and are completely sufficient** to achieve the goal of the development. They are presented clearly, in the appropriate sequence. The data are very well summarized, systematized and presented in the "Results" section, being reflected in **27 tables** from which the obtained data can be clearly seen and compared. They are illustrated with **14 figures**, which include 6 good quality photographs. They are well formed technically and aesthetically. The charts further illustrate the ratios of the microorganisms isolated from the milk samples from the surveyed farms and aid in the clear presentation and comparison of the data. The text in this section further explains the results in a comparative aspect. From the way of presenting, describing and comparing the obtained data, it is evident that the author has mastered the applied research methods, as well as the processing and competent analysis of the results. A certain inconsistency is noticed in some of the numerical data and the degree of credibility indicated in the tables 20 - 22 and in the text to them, as well as in table 24 in terms of confidence level in differences in neutrophils. Part of the data from the first paragraph of the last section of the results concerning the

thermographic diagnosis of subclinical mastitis (p. 119 below and 120 above) is duplicated with that of the corresponding section in "Materials and methods" (p. 75, third paragraph). The relevant text referring to the device and method description should be dropped from the results. The text explaining the results presented in Figures 13 and 14 should indicate that the reported temperatures are in degrees Celsius.

In this section of the dissertation, the data obtained from the conducted researches are fully presented, which shows the high competence of the author and his ability to apply the learned methods, as well as to summarize, present and compare the obtained results. They show that **the set tasks have been completed and the goal of the work has been achieved.**

• **Discussion of the results and used literature.**

The Discussion section is also interesting, competently and engagingly written. It presents an in-depth interpretation of the obtained results, an analytical comparison with those of other authors, explanations of the reasons for the differences found, and the author makes reasonable assumptions in this aspect. A full analysis of the data from the complex studies was made, and here the high literary awareness of the doctoral student and his ability to interpret and compare the results also stand out. In this section, instead of "These results of ours for are also supported by other authors" or "These authors find similar to ours", it is appropriate to write that "our results for support these and other authors' or 'We find similar to that of these authors', following the chronology of the compared studies. The taxon "Dermacoccaceae" (p. 116 below) is not a genus but a family. Some parts of the text in this section constitute a literature review, such as the last two paragraphs on page 135, which are analogous to the data on page 50 of the literature review.

The literary index includes 254 sources and shows the author's good awareness of the state of the problem in our country and abroad. Eight of the sources are in Cyrillic and the remaining 246 are in Latin. The share of sources from the last 10 years is significant – 37% (94 items), which is a testimony to the **good awareness of the author at a modern level.** Sources from the period 2000-2012 predominate (52%, 131 items), and the least (11%, 29 items) are those from before 2000. The bibliography is well designed according to standard requirements.

As a result of the studies carried out, **6 conclusions** were formulated, which accurately and synthesized reflect the most important of the obtained results in the sequence of the set tasks.

• **Contributions of the dissertation.**

The contributions resulting from the research and analysis carried out are **seven** in number, clearly formulated. They are scientific and applied. **Three of them are original for our country**, and the remaining four are confirmatory. The microorganisms causing the subclinical mastitis in sheep in our country have been identified, as well as their sensitivity to antimicrobial agents. For the first time in our country, the possibility of using non-contact thermography for diagnosis of these diseases in sheep has been studied. In addition, the humoral and cellular defense mechanisms of the mammary gland in ewes with subclinical mastitis were investigated. The spread of these mastitis in lactating sheep in our country has been determined. The cut-off value of 500,000 somatic cells in 1 ml of milk has been determined as appropriate for the diagnosis of subclinical mastitis in sheep. The changes in the physicochemical indicators of milk in these diseases have also been established. It has been shown that the major immunoglobulin in blood serum and milk is IgG, and its values, as well as milk lactoferrin and lysozyme levels, are significantly increased in sheep affected by subclinical mastitis.

Two recommendations for practice have been formulated, the application of which would be useful in the diagnosis and therapy of subclinical mastitis in sheep in our country.

• **Assessment of the degree of personal participation of the dissertation author in the contributions.**

As I have witnessed the doctoral student's work on the dissertation, I would like to highlight his active participation in all stages of its development. He exerted maximum effort and showed particular diligence, discipline and responsibility in surveying the farms, obtaining the samples,

conducting the research, reading, summarizing and analyzing the results, in writing this work and the publications related to it. **It is his own work**, carried out under the expert guidance of his scientific supervisor.

- **Critical remarks, recommendations and questions.**

My critical notes and recommendations on the project of the dissertation were presented to the author in advance, and some of them to the Extended Departmental Council during the preliminary consideration of the dissertation. They were taken into account by the author to the maximum extent. In the previous sections of this opinion, I have mentioned **some technical errors and omissions, which, however, in no way detract from the value and importance of this work**, and the purpose of mentioning them is to avoid them in future publications.

- **The abstract is well-formed technically and aesthetically. It objectively reflects the structure and content of the dissertation work.** It is written on 38 pages and fully presents the information and research results described in the dissertation. It includes the necessary sections according to generally accepted requirements, as well as 27 tables and 12 figures presenting the results.

- **Published articles and citations. Evaluation of the dissertation publications. Reflection in science - use and citation by other authors.**

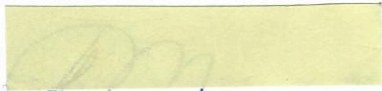
The attached list of publications related to the dissertation includes **two** articles, shown in the penultimate section of the dissertation. They are in English, and in one of them Assistant professor Alexander Stoimenov is an independent author and in the second one he is the leading. They are printed in 2022 in the scientific journal of FVM, UF "Tradition and modernity in veterinary medicine", which is referenced in CABI - Web of Sciences. **These scientific publications are sufficient for the defense of the dissertation.** For this short period, there is no information about citing these articles by other authors.

In conclusion, I declare that **the dissertation work presented by Assistant professor Alexander Stoimenov meets the criteria and indicators for the acquisition of educational and scientific degree "Doctor" set in the Law on the Development of Academic Staff in the Republic of Bulgaria, the Rules for its implementation and the Rules for development of academic staff at University of Forestry in Sofia.** The topic is relevant and has a valuable practical focus. The author's literary awareness is very good, as is his ability to use literary information in his research work. The dissertation is sufficient in volume and well constructed according to the requirements. Scientific publications related to it are also sufficient to acquire this educational and scientific degree. Research has been carried out at a modern level, and the obtained results are clearly presented and competently analyzed. Practically useful contributions originate from them. Based on the presented information and its analysis, as well as my personal impressions, I express my conviction that Assistant professor Alexander Stoimenov is an excellently prepared and promising young specialist with in-depth theoretical and practical training in the scientific specialty, with the skills to apply modern scientific methods research, as well as to process, compare and interpret the obtained results.

All this gives me the reason to express my positive vote and suggest to the respected members of the Scientific Jury to also **vote positively for the acquisition by Assistant professor Alexander Alexandrov Stoimenov of the educational and scientific degree "Doctor" in the scientific specialty "Obstetrics and gynecology of animals and diseases of newborn animals"**, field of higher education 6.0 Agricultural sciences and veterinary medicine, professional field 6.4. Veterinary Medicine.

30.12.2022
City of Sofia

Opinion prepared by:


(prof. T. Popova, DSc)