

REVIEW

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Regarding: Dissertation work on the topic "ANALYSIS OF SELECTION CRITERIA FOR NATURAL NEMATODE RESISTANCE IN SHEEP", with author Victoria Emilova Marincheva and scientific consultant Assoc. Prof. Dr. Andrey Kurtenkov, for the award of the educational and scientific degree "Doctor", in specialty "Breeding of agricultural animals, biology and biotechnology of reproduction", field of higher education 6.0. "Agricultural Sciences and Veterinary Medicine", professional field 6.4. "Veterinary Medicine".

This review was prepared on the basis of participation in a scientific jury, appointed by order of the Rector of LTU No. ZPS - 561/14.11.2023.

BRIEF BIOGRAPHICAL DATA

Victoria Emilova Marincheva was born on 02.12.1983 in the city of Sofia. In 2002, she completed her secondary education at 81 "Victor Yugo" Secondary School, Sofia, and in 2008 she graduated as a Master of Veterinary Medicine at the Forestry University. Victoria Marincheva worked as a veterinarian at the Central Veterinary Clinic - Sofia from 2009 to 2016, and from 2016 to 2020 - at the "Animal Rescue Sofia" Foundation, and during this period continued to develop her professional knowledge and skills, going through a number of courses, internships and scientific events at home and abroad (Acupuncture Course at National Sports Academy and Jiangxi University of TCM – China; Internship in Veterinary Neurology and Neurosurgery at Small Animal Referral Neurology Clinic, Helsinki, Finland; Internship at Multidisciplinary Veterinary Clinic Citta di Pavia, Italy; Volunteer Castration Program of stray dogs of the Four Paws Foundation; Veterinary echography and echocardiography courses at the Central Veterinary Clinic - Sofia; World Leish-6 Congress, Toledo, Spain; Eastern European Regional Veterinary Conference, Bucharest, Romania). Since October 2020, Viktoria Marincheva has been an assistant in the Department of Anatomy, Physiology and Animal Husbandry Sciences of the Forestry University, and in March 2023 she has been enrolled in doctoral studies at the same department in the specialty "Breeding of farm animals, biology and biotechnology of reproduction". The doctoral student has fully fulfilled the minimum national requirements for obtaining the educational and scientific degree "Doctor" in a field of higher education 6.0. Agricultural Sciences and Veterinary Medicine, professional field 6.4. "Veterinary Medicine".

ACTUALITY OF THE SUBJECT

Nematodoses in animals, in particular sheep, are among the most widespread parasitoses, which cause serious damage to their productivity, and their control faces a number of difficulties. In this regard, research contributing to combating the

problem deserves close attention. The dissertation aims to carry out studies that would support the fight against nematodes in sheep at a new level - not with drugs, but with prevention in a natural way, by trying to study criteria for the selection of sheep resistant to infestations with gastrointestinal nematodes. The development of such a work in the era of global spread of resistance to drugs, in particular anthelmintics, makes the topic particularly relevant.

KNOWLEDGE OF THE PROBLEM AND LITERARY AWARENESS OF THE CANDIDATE

The good literary awareness of the candidate is evident from the "Literature overview" section. Victoria Marincheva introduces the topic of the dissertation by starting from afar - with a description of the knowledge on sheep domestication and the state of sheep breeding in Bulgaria. She then introduces the reader in detail to the studies carried out to date concerning various aspects of the subject: economic losses from nematodes in sheep; the etiological, epidemiological, pathogenetic, clinical and diagnostic aspects of gastrointestinal nematodes in sheep; the anthelmintic drugs used in the fight against nematodes; the mechanisms for the development of anthelmintic resistance and immune defense during invasion with gastrointestinal nematodes; the parasitological and clinical methods for evaluating gastrointestinal nematode infections in sheep; selection for resistance to gastrointestinal nematodes as an alternative to drug control; related genomic studies and practices for integrative control of gastrointestinal nematode infections. The PhD student cites leading research in the field, through which she clarifies key concepts for the dissertation, such as anthelmintic resistance, tolerance, resilience, refugia, hypobiosis, heritability, immunonutrition, self-healing, selective breeding and others.

A total of 395 sources are cited in the dissertation, most of them (378) are in Latin, 17 are in Cyrillic, and 200 refer to research carried out after the year 2000. All this shows that Marincheva has fully familiarized herself with the researched problem, which gives her solid theoretical training on the different aspects of the developed topic.

STRUCTURE OF THE DISSERTATION

The dissertation is written on 195 pages and is structured as follows: Title page; Contents - 3 pages, List of abbreviations - 1 page, Literature review - 54 pages, Aim and objectives - 1 page, Materials and methods - 13 pages, Results - 49 pages, Discussion - 38 pages, Conclusion - 3.5 p., Conclusions - 1.5 p., Contributions - 1 p., Recommendations for practice - 1 p., Cited literature - 32 p. and List of publications on the topic of the dissertation. The work is illustrated with 74 tables and 16 figures, accompanied by the necessary documentation, including an autoabstract.

COMMENTS ON THE DISSERTATION

Title

The title of the scientific work is short and clearly reveals its essence.

Literature review

As I have already indicated the Literature Review section is comprehensive and provides up-to-date and detailed information on various aspects of the development. Regarding the section, I express the following recommendation: It would be good to make a short summary at the end of it, through which to motivate the need for conducting the research and then move on to the aim.

Aim and objectives

The study is aimed at analyzing criteria for selection of sheep based on natural resistance to gastrointestinal nematodes and evaluating their applicability in practice. For its implementation, 9 specific tasks have been set, which are clearly formulated. The set goal and tasks correspond to the title of the dissertation.

Materials and methods

The research has been carried out in 2021 on 46 dairy sheep, which were divided into 3 groups (25 ewes, 9 breeding rams and 12 young female sheep), randomly selected from a herd located in a registered animal breeding site in the territory of Haskovo region. After initial clinical examinations according to a standardized protocol in order to unify the animals in the experimental groups, they have been repeatedly subjected to hematological (examination of complete and differential blood count, biochemical blood examination, examination of electrolyte content), parasitological (quantitative coproovodiagnostic by modified Mak Master method) and specific tests (exterior measurements, assessment of body condition - BCS, assessment of the degree of fecal pollution - Dag score, assessment according to the FAMACHA system). The doctoral student has described in detail the apparatuses, reagents and the sequence of performing the research and illustrated the section with three figures and one table. The results of the conducted research have been statistically processed and subjected to regression analysis. The following interrelationships were followed: between the indicators of the red blood cell count and the number of parasitic eggs in one gram of faecal mass (FEC), between the FAMACHA score and FEC, between some external measurements, the body condition score and FEC, between the degree of faecal contamination and FEC, between birth order and FEC, between animal sex and FEC. Climatic factors in the area of the conducted research in the sampling period are reported. The materials and methods used are relevant to the purpose of the study.

Results

The data in this section are described in 15 points: Clinical Examinations, Ewe Infestation Monitoring, Breeding Ram Infestation Monitoring, Young Female Sheep Infestation Monitoring, Exterior Measurements, Body Condition Evaluation, Grade Evaluation of faecal contamination, Evaluation according to the FAMACHA system, Accounting for the order of lambing in ewes and other dependencies, Hematological studies, Biochemical studies, Differential blood count and percentage of eosinophils, Albumin:globulin ratio, Statistical analysis and Meteorological data. Most of the results are presented in tabular form (from Table 6 to Table 63), reflecting the

average, minimum and maximum values of the various indicators during each reading for the different categories, as well as the individual values for each animal.

The results regarding the degree of parasite infestation reflect the FEC values in the different categories of animals, with ewes showing data from three readings (in the months of February, April and May), rams from two readings (in April and May) and young female sheep – from one reading (in May). Exterior measurements have been performed once on the ewes at the beginning of the experimental period; evaluations of the body condition, the degree of contamination with faeces and according to the FAMACHA system were made twice for the ewes (February, May) and rams (April, May) and once for the young female sheep (May).

Marincheva shows the results of the performed hematological tests in two tables. She comments on the values of various indicators in individual animals, from which it can be seen that most fall within the reference limits. Abnormalities such as low-grade anemia, hypochromic anemia, thrombocytopenia, and thrombocytosis have been found in some animals.

The doctoral student presents the results of the biochemical blood tests in separate tables regarding the indicators related to the function of the kidneys and liver, the metabolism of carbohydrates, proteins and fats, as well as the content of minerals in the blood. The values for most parameters in the ewes were within the normal range, except for the total bilirubin content, which was above the reference values in the majority of animals, and globulin content, which was also above the upper limits in some animals. Bilirubin has been also higher in many of the rams and young female sheep, and globulin has been higher in some of the rams. Results above the reference values have been found for aspartate aminotransferase in rams and young female sheep and for total protein for a large part of rams.

The subsection "Statistical analysis" includes 12 tables that show what correlation coefficient has been found in the different categories of animals between the following indicators: FEC and those from the complete blood count; FEC - creatinine and urea; FEC - alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase, total bilirubin; FEC - albumin, total protein and globulin; FEC - percentage content of eosinophils; FEC - albumin:globulin; FEC - assessment according to the FAMACHA system; FEC - body condition assessment; FEC and BCS in ewes - some exterior measurements; FEC - degree of fecal pollution; FEC - birth order. A table summarizes the percentage of ewes and rams with low and high FEC over the two reporting periods.

At the end of the "Results" section, the climatic features of the area where the sheep flock included in the study is located and the meteorological data for the days during which materials for the development were collected are described.

Discussion

The section follows the logical order of the tasks set and the results presented. It begins with a discussion on the infestation degree of the researched animals during the different stages of the study. The doctoral student subsequently comments on the results of the ewes, the rams and the young female sheep, illustrating them appropriately with five graphs. She explains the high levels of FEC in the month of February in the ewes and the statistically significant decrease in the indicator in the following two studies with the critical perinatal period during which the immune response is lowered and its gradual recovery afterwards. Marincheva points out that

the assessment of this indicator in rams is even more important, because the choice of a breeder with good genetic potential is the basis for improving the qualities of the entire herd. The PhD student examines the results regarding the degree of parasitizing individually for each specific animal, indicating at which FEC indicators and accompanying factors do or do not require deworming and which animals should be subject to positive selection.

Further, the discussion is focused on the analysis of external measurement indicators, body condition assessment, assessment of the degree of faecal contamination, FAMACHA system assessment and indicators of general and biochemical blood tests. The interpretation of hematological parameters is adequately supported by eight tables and that of the other study-specific parameters by three graphs. The next criteria that are discussed are the interrelationship between Dag score and FEC, between birth order and FEC, between animal sex and FEC. According to Marincheva, there is a correlation between FEC and Dag score, but it is not well expressed in all animals during the different periods, therefore the evaluation of body condition can be used as a selection criterion, but only in combination with other indicators and in judgment on an individual level. The PhD student also discussed possible reasons for the lack of a clear relationship between animal age and FEC, as well as the relatively higher susceptibility of male animals to infection with gastrointestinal nematodes.

The final subsection, Analysis of Climatic Factors in the Sampling Period, is informative rather than debatable. Most of the information it contains should be placed in the introduction of the dissertation.

Conclusion

The conclusion is quite extensive, disparate information is presented in it, much of which should be placed in the results, discussion and conclusions sections of the dissertation.

Conclusions

Nineteen conclusions have been drawn. They reflect the results of the dissertation work, but their wording needs refinement. Some of these are quite descriptive and are presented more as summary information from the results and discussion. It would be better to reduce the number of conclusions, for example with combining some conclusions concerning a common criterion.

Contributions and recommendations for practice

From her dissertation work, Victoria Marincheva formulated 13 contributions and 8 recommendations for practice. I agree with the characterization of the first 5 contributions as original for Bulgaria, and the other 8 as confirmatory. Among them, those that document the possibility of selecting sheep for resistance to gastrointestinal nematodes based on the criterion of the number of parasitic eggs per gram of faecal mass stand out, as well as those that indicate the importance of the FAMACHA and Dag score systems as additional in this respect. The recommendations for the practice arise from the overall analysis of the own results and previous scientific research in the direction. They are up-to-date, accurate and

following them would take control of gastrointestinal nematodes in sheep to a new level.

Scientific publications and autoabstract of the dissertation work

Victoria Marincheva presents 3 articles in connection with the dissertation, one of which is an overview. Two of the articles are published in the journal *Zhivotnovadni Nauki*, of the first one the doctoral student is an independent author, and the other article is co-authored with her scientific consultant. The third article is co-authored and published in the *Global Journal of Animal Scientific Research*, with Marincheva as the first author. Both journals are international, indexed in a number of databases such as CABI, PubMed Resources, AGRIS, EBSCO, etc.

The content of the autoabstract corresponds to the material presented in the dissertation. It is written on 43 pages and contains 58 tables.

NOTES AND RECOMMENDATIONS

The doctoral student complied with a large part of the recommendations that I made during the preliminary discussion of the dissertation work by the extended departmental council. At the moment, I could make the following notes and recommendations, which are mostly of a technical nature:

- The dissertation does not have a separate "Introduction" section. The first paragraph included in the literature review appears with a such function, but it should be presented as an independent part in the structure of the development.
- The abbreviated terms BCG, DGKC, IFCC, DMSO, GOD-PAP, OCPC, UV (pages 58-59) are not included in the list of abbreviations. The full names of the biochemical blood indicators are indicated in the "Materials and methods" section, it would be more correct to include them in the list of abbreviations.
- Only the Latin names of the species and genera of the parasites should be written in Italics. The names of families and higher taxa are written in plain type, "species" (sp., spp.) is also written in regular font after the generic names.
- Table 29 is entitled "Investigation of Cholesterol (Chol) and Triglyceride (TG) parameters in rams" but refers to results in ewes.
- When describing the results of biochemical blood tests, calcium, phosphorus and magnesium are indicated as macroelements, and potassium and sodium as electrolytes. In this case, there is no need to separate them, since they are all both macroelements and electrolytes.
- Indicators related to fat metabolism in rams and young female sheep (pages 99,104), glucose in young female sheep (page 102) and minerals in blood in young female sheep (pages 104-105) are not accompanied by reference values and are not commented on whether they were normal.

MOTIVATED ANSWER TO THE QUESTION WHETHER THE DISSERTATION RESEARCH IS MAINLY THE PERSONAL WORK OF THE DOCTORAL STUDENT

Viktoria Marincheva is a PhD student at self-study training. From the dissertation work and its accompanying documentation, as well as from her presentation during the discussion of the dissertation project in front of the

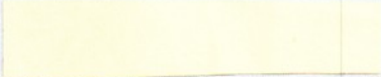
Department of Anatomy, Physiology and Animal Breeding Sciences of the FVM at LTU, it is clear to me that she has been active and has had a leading involvement during all of the research stages. The publications related to the dissertation testify that Marincheva has developed qualities such as initiative, independence and, at the same time, good teamwork skills. All this assures me that the dissertation research is primarily her own work.

CONCLUSION

The dissertation examines an actual topic, it is innovative for Bulgaria, accompanied by a rich literature review, its purpose and tasks are correctly set and it is prepared with an appropriate methodology. The results obtained, their interpretation and the conclusions drawn have value both for parasitology and for sheep breeding. The research indicates available criteria such as FEC, BCS, FAMACHA, Dag score, standard blood count and biochemistry, which can be successfully used in evaluating sheep for positive selection based on natural resistance to gastrointestinal nematodes.

The circumstances listed above and the documents accompanying the dissertation show that the goals of the educational and scientific degree of the doctoral studies have been met. This gives me the reason to vote positively for awarding Victoria Emilova Marincheva the "PhD" degree in the scientific specialty "Breeding of agricultural animals, biology and biotechnology of reproduction", field of higher education 6.0. "Agricultural Sciences and Veterinary Medicine", professional field 6.4. "Veterinary Medicine".

06.02.2024
Sofia

Reviewer : 
/ Assoc. Prof. Dr. Mariana Panayotova-Pencheva /