# REVIEW

by Prof. Dr. Lilyan Krumov Sotirov, Dept. Animal Genetics, Faculty of Veterinary Medicine, Trakia University, Stara Zagora

of a dissertation work "Analysis of selection criteria for natural resistance to nematodes in sheep" with author Assistant Professor Viktoria Emilova Marincheva and scientific supervisor Associate Professor Andrey Kurtenkov, PhD. Field of higher education: 6. Agricultural sciences and veterinary medicine, Professional direction: 6.4. Veterinary Medicine Scientific specialty: "Breeding of agricultural animals, biology and biotechnology of reproduction".

**Biographical data**

Assistant Victoria Marincheva was born on 02.12.1983 in the city of Sofia. Primary education is completed in 144 Secondary School "Narodni Buditeli", a class with extended training in music and piano, and on average in 81 Secondary School "Victor Yugo", a class with extended training in English and German. She completed her higher education in 2008 at the Forestry University, Sofia, majoring in "Veterinary Medicine". From 2009 to 2016, she worked as a veterinarian in a multidisciplinary clinic for small animals, with the main areas of responsibility being internal medicine and cardiology. In 2016-2020, she was the manager and chief physician of the "Franciska" veterinary clinic at the Animal Rescue Foundation, Sofia. In 2020, after winning a competition, she started working at LTU, FVM, Department of Animal Science, as an assistant in the disciplines of Genetics and Animal Breeding, Animal Husbandry and Canine Science.

The thesis contains 195 standard typewritten pages that include text, tables, diagrams, photographs, and a bibliography. 392 authors are cited, of which 17 are Cyrillic and the rest are Latin. The literature review is quite detailed and is presented in 13 points. The information presented is intended to provide insight into the research on the indicators of interest in this dissertation project. Based on the research done, the author and her supervisor set the goal of the dissertation work to analyze some selection criteria based on natural resistance to gastrointestinal nematodes in sheep and to evaluate their applicability in practice.

For the fulfillment of this goal, 9 tasks have been formulated:

1. Determining the health status of the herd.
2. Carrying out laboratory blood tests.
3. Conducting parasitological studies:
4. Measurement of some exterior indicators.
5. Body condition scoring (BCS).
6. Assessment of contamination with feces (dag score).
7. Introduction of the FAMACHA system (system for grading the severity of color chart anemia).
8. Establishing a relationship between the studied indicators and statistical analysis.
9. Analysis of climatic factors in the sampling period.

A total of 46 experimental animals were used for the implementation of the project, of which 25 ewes, 9 breeding rams, and 12 gilts. Individuals were randomly selected from a herd of about 800 animals, of which approximately 450 ewes, 15 breeding ewes, and 250 whistles. The experiment

was carried out in 2021. The herd is located in a registered animal breeding site in the territory of the Haskovo region and consists of milk breeds, with a large part of the population including the Asaf breed of the fifth generation after crossing with local sheep, a few animals of the Avasi breed ( mix or purebred) and one Lacon ram.

An Individual Animal Research Protocol was used. The reagents, laboratory equipment, and apparatus used are described in detail. It is also indicated which clinical, biochemical, and hematological signs were investigated. To fulfill the set tasks, seven biological experiments were carried out. The results of the experiments were subjected to statistical analysis with Microsoft Excel 2016. Mean values with standard deviation ($X \pm SD$), minimum and maximum value, and correlation coefficient calculated using the CORREL function and statistical significance ($p < 0.05$) based on regression analysis. Part of the data is presented as a percentage for a clearer understanding of the results. The obtained results are presented as follows:

**First biological experiment:** Tracking the degree of parasitism in ewes from the postpartum period until the time of an expected increase in the number of ewes. Three studies were performed in February, April, and May 2021. Results are presented as arithmetic mean ± standard deviation (SD). It is noteworthy that the SD has higher values than the arithmetic mean, which is strange, and if the numbers are correct, this indicates a huge variation of the studied trait, which in turn requires a serious analysis of the obtained results.

**Second biological experiment:** Tracking the degree of parasitism in rams breeders. The results are like the first biological experiment.

**Third biological experiment:** Investigation of the level of parasitism in buzzards. Same result.

Fourth biological experiment: Measurement of some exterior indicators. The measurement was made with a ruler in an upright position of the body. A stretch index and compactness index were calculated, which give additional information on body conformation in a category of ewes. The results are presented in the Table. 7

**Fifth Biological Experience:** Body Condition Score (BCS). The results are presented in the Table. 8, 9 and 10.

**Sixth biological experiment:** Assessment of the degree of fecal pollution (Dag score). The results are presented in the Table. 11, 9 and 13.

**Seventh biological experiment:** Evaluation according to the FAMACHA system.

Consideration of lambing order in ewes and other dependencies: Descriptive and relevant to the discussion.

**Hematological and biochemical studies**

Table 17 presents Reference values of hematological indicators in sheep from different sources. For this study, only the reference values for the Mindray BC-2800 Vet analyzer are relevant, because the study of the experimental animals was carried out with this apparatus. The remaining reference values could be used in the discussion. All hematological tests on ewes carried out in February and May are within the limits of the quoted reference values. The results are similar for the rams and whistles. Biochemical parameters creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, total bilirubin, glucose,

albumin, total protein, globulin, urea, cholesterol, triglycerides, calcium, phosphorus, magnesium, potassium, sodium, creatinine in sheep, goats and rams correspond to the reference for the Mindray BA-88A Biochemical Analyzer.

**Differential blood count and percentage of eosinophils**

The preparation of a differential blood count with the calculation of the percentage of eosinophils in the peripheral blood aims to look for a relationship with the FEC result. For this reason, smears were made every time a fecal sample was taken. Mean values in ewes are calculated as follows: 3.3 ±2.09 % for 27.02.2021, 4 ±2.06 % for 4.04.2021, 6.25 ±4.16 % for 9.05.2021. Eosinophil percentage varies from 0 to 9% on 02/27/2021, from 1 to 8% on 04/04/2021, and from 1 to 17% on 05/09/2021. The average values for rams are equal to 5.63 ±2.6 % on 4.04.2021 and 3.13 ±1.69 % on 9.05.2021 d. The limits of the indicator are from 0 to 11 %. The percentage of eosinophils is 4.66±1.88 % with a minimum value of 2% and a maximum of 7%.

**Albumin: globulin ratio**

For the group of ewes, the mean albumin: globulin ratio was 0.78 ± 0.11 on 02/27/2021 and 0.68 ±0.1 on 05/09/2021. For rams, the average value is equal to 0.84 ± 0.22 on 04/04/2021 and 0.79 ± 0.17 on 05/09/2021. For whistles, the average value is equal to 0.83 ± 0.13.

**Statistical analysis**

A correlation was established between the parameters of the red blood cell (erythrocytes, hemoglobin, hematocrit) and the values obtained at FEC, which varied from weak to high with a positive or negative sign. The results for the association between FEC and biochemical indicators are similar. The correlation between FAMACHA score and FEC ranged from moderate to high. The correlation between some body measurements and FEC varied from weak in ewes to high in rams, but was always negative, meaning that large, well-developed animals had low parasite egg counts. The relationship between Dag score and FEC varied from weakly negative in rams to high in ewes and whistles. The firing sequence does not affect the FEC values, which is reflected in the low correlation coefficient. The percentage of HIGH and LOW FEC animals in ewes and rams ranged from 12% to 56%. Meteorological data for the time of the experiment are also presented.

**DISCUSSION**

In this section, the obtained results are analyzed. According to the author, the selection of breeding animals that meet certain qualities is an important principle in breeding practice and aims at sustainable transmission to the offspring. In the ewes in the first two studies, the highest percentage of animals with medium to high FEC (EPG 250-750) was 44%, and these values decreased to 32% at the end of the experimental period. Animals with high FEC (EPG above 750) covered 36% in the postpartum period and subsequently decreased to 20% and finally to 12%. In animals with low FEC, the percentage gradually increased: from 4% to 8% and 28% with EPG 50 at the beginning of May, and from the beginning of April 4% with EPG 0 was established. In rams, unlike ewes, it was established constant percentage of individuals with low FEC - 33%. The percentage of those with high FEC increased, from 11% to 44%. In the case of buzzards due to the deworming carried out on February 10, 2021, the results cannot be considered objective. According to the author, immunological reactivity ensures the expulsion of the adult forms and suppression of the fecundity

of the female parasites. The phenomenon of self-healing is known, in which the larvae or adult stages are expelled from the body without requiring the use of drugs. Expulsion is based on hypersecretion of mucin, increased concentration of histamine and leukotrienes, and also on enhanced peristalsis. An individual's ability to limit or reduce parasite load is part of the resistance phenomenon. Another important category is ewes showing a consistent LOW FEC or below 250 EPG. The LOW FEC trait team is widely used especially in the experimental and commercial herds of Australia and New Zealand. From an economic point of view, this means fewer losses due to reduced productivity, illness, medication use, and in some cases even death. Breeding of herds resistant to SFN is assessed as imperative worldwide due to the reduced effectiveness of known dewormers and the demand for foods with limited content of chemical residues. When looking at MEDIUM FEC ewes (250-750 EPG), an assessment should be made to what extent parasite infestation affects general condition and productivity. It turns out that sheep with consistently HIGH FEC (over 750 EPG) are fortunately not many. If these animals do not exhibit some other extremely valuable qualities that should be preserved, then they should be scrapped as the main propagators of SCN, which will also require costs for dewormers and are likely to reduce their productivity.

The relationship between deworming and FEC in ewes was also investigated. It was established that, despite the deworming performed in the postpartum period, high EPG values (over 750) were found in a significant part of the animals (9 animals). It's a similar analysis also made for rams emphasizing that the selection of resistant breeders is very important because these breeders have a serious effect on their progeny in terms of parasite resistance. For selection, those with consistently LOW FEC are most important. At HIGH FEC, selection becomes negative, and such individuals should be scrapped.

**Exterior indicators and indices**

It was found that the body dimensions and the indices derived from them show a relatively good evenness of the herd. This, in turn, reflects the conditions of rearing, the degree of protection and the health status of the animals.

**Body condition assessment**

The author states that the general body condition of the sheep is directly related to the degree of worming, citing specific examples. Sheep #1 showed a significant decrease in FEC from 900 to 50 EPG and a corresponding improvement in BCS. In ewes #9 and #20, which show HIGH FEC throughout the period, the BCS is lowered to 2. According to the presented results, BCS in rams shows higher levels, which are explained by the large body and better muscularity in the male sex. The animals are in very good condition and the BCS score is equal to 3, and for coach No. K2 to 3.5 for both dates. Koch No. K4 rated 3 on 4.04. and 2.5 on 9.05. an increase in FEC from 700 to 2650 EPG is reported, while the FAMACHA score changes from 3 to 4.04. on 4 on 9.05. The body condition of test animals of this group is considered satisfactory in the case of guinea fowl.

**Assessment of the degree of fecal pollution (Dag score)**

In the assessment of the Dag score, a significant deterioration of the indicator is found when the sheep, rams, and heifers leave the pasture. The author rightly explains that, in addition to worming,

this indicator is strongly influenced by other factors such as fungal endophytes, diet and physiological state.

**Evaluation according to the FAMACHA system**

According to the author and other quoted authors, this evaluation system is quite subjective because it depends on the condition of the examinee, the lighting of the room, or the sunlight. In the herd, at least in the opinion of the author, the normal color of the conjunctiva in clinically healthy animals corresponds to 3 according to the FAMACHA system. According to the literature, such animals should be examined regularly, especially if they fall into the category of lambs and lactating sheep, and deworming is applied at the first signs of disease.

**Hematological and biochemical studies**

As stated in the "Results" section, in most animals, the hematological and biochemical indicators are the limits of the reference values established for the biochemical analyzer used. Abnormalities found in individual animals may be due to an inflammatory and/or infectious process - for example, foot rot, which affects a large part of the population used for experiments and animals with pronounced lameness were excluded at the very beginning of the studies; another cause may be subclinical mastitis, etc.

**Indicators related to kidney function.**

The values of the creatinine indicator are within the reference limits, which confirms preserved renal function in all studied groups. It has been established that the excretion of this metabolite does not depend on the type of feed intake. The correlation coefficient concerning FEC is equal to 0.036 for ewes, - 0.084 for rams, and 0.026 for gilts. Since the results are close to zero, no correlation between the two indicators can be deduced.

**Indicators related to hepatic metabolism.**

Examining these indicators can be a good indicator of stress, including that caused by SFN worming. Authors cited as indicating that liver enzymes are seriously affected by season and degree of deworming. The lack of generally valid reference values in sheep necessitates working with the limits determined by the specific laboratory and the equipment it has available.

**Indicators related to carbohydrate metabolism and protein metabolism.**

The results obtained from this study for the different categories of animals are within the reference values determined for the equipment used. It is important to emphasize the preserved protein balance against the background of an established parasite infestation. Animals can tolerate deworming without developing hypoalbuminemia or hypoproteinemia. Even in the HIGH FEC subjects, the levels remained like those of the other subjects in the experimental group. Hypoalbuminemia can be expected with protein deficiency and cachexia. In all three categories of animals, no correlation was found between FEC and globulin concentration. However, the correlation between the levels of total protein/albumin and total protein/globulins. The inferred correlations presented in Tables 54 and 55 in the results section show that there is a statistically significant relationship between FEC/total protein/globulins in the peripartum period in ewes when the highest levels of parasite infestation are accounted for. At a later stage, this relationship is less pronounced. In the case of rams, this attitude is more clearly expressed in the studies on 9.05.,

which coincides with an increase in the number of parasitic eggs. The correlation is weak and with a negative sign in the case of whistles. It can be concluded that the levels of total protein and globulins show a positive correlation with FEC only at high EPG values, but this relationship is not constant and cannot serve as an indicator in the analysis of the clinical condition of animals infected with SFN.

**Indicators related to the metabolism of fats, macro and microelements.**

The values of these indicators for most animals from the three categories are within the reference values typical for the equipment used.

**Percentage content of eosinophils and FEC**

Eosinophilia is an indicator of the body's reactivity to parasitic invasion. Sheep selected for resistance to *Haemonchus contortus*, are characterized by an increased percentage of eosinophils. In experimental infection of lambs with Trichostrongylus colubriformis, the level of eosinophilia was used as an indicator of the development of host resistance. Based on the obtained results in case of natural infection with SFN, the opinion can be expressed that there is no clear relationship between the percentage of eosinophils in the peripheral blood and FEC. To increase the reliability of the conclusions, a differential blood count was performed at each fecal sample collection. However, a large part from the experimental data of other authors cannot be confirmed. An analysis of the obtained results with those of other authors was made.

**Correlation between RBC parameters (erythrocytes, hemoglobin, hematocrit), FAMACHA and FEC values**

Ewes:

In ewes, the correlation coefficient between FEC values and the FAMACHA score of 27.02 was equal to 0.4 (0.416); at 9.05 the coefficient increased to 0.8 (0.813), which was due to a decrease in the number of parasitic eggs while maintaining the FAMACHA score for the group. These values are close to + 1 and give a statistically significant relationship between the studied characteristics. However, when a comparison is made at the individual level, the results are not as clear-cut, especially when blood tests are added to the assessment. In my opinion, this should not be done, because statistical indicators characterize the sample, and through it the general population as a whole, and they cannot characterize the individual unit. Conclusions are made on the basis of average statistical indicators and not on individual results. The results are similar in the case of rams and dzvizkas.

**Correlation between Dag score and FEC**

At the Dag score evaluation of the ewes at the beginning of the experimental period (27.02), the animals were classified as 1 (52%) or 2 (48%), and the faeces were mostly formed and less often soft unformed. Based on the presented data, the author concludes that there is a correlation between Dag score and FEC - with a higher Dag score, a higher FEC is also expected; this is true for 48% of ewes. A correlation coefficient of 0.7 (0.698) is found, which indicates a significant statistical dependence. At the end of the experimental period (9.05), the following distribution is calculated: 20% with Dag score 2, 56% with Dag score 3 and 24% with Dag score 4. The correlation coefficient is 0.3 (0.275) and accordingly the relationship between the two indicators becomes

significantly lower. Here again, it should be emphasized that the Dag score depends on several other factors and the most likely reason for the increase of the indicator is fresh grazing in the spring season. When comparing the Dag score data on 27.02. and 9.05. a statistically significant difference was found in the ewes ($p < 0.05$). Dag score with most rams at 4.04. is 2 with feces mostly formed and FEC ranging from 50 to 800 EPG.

**Establishing a relationship between the order of loading and the FEC**

The correlation coefficient between the indicated signs has a negative value: - 0.005 compared to FEC on 27.02. and - 0.05 vs. FEC of 4.04. and 9.05, which means there is no connection between them.

**Establishing a relationship between gender and FEC**

A permanent decrease in the average number of parasitic eggs was observed in the ewes and an increase in the rams without deworming. Based on these data, the author expresses the opinion that male animals are relatively more sensitive to infection with SCN. According to the cited authors, this could be explained by the immunosuppressive effect of testosterone and the higher consumption of weed.

**Analysis of climatic factors in the sampling period**

The author establishes a connection between the climate and the parasitization of animals, which is in sync with the cited authors, according to whom warming provides better opportunities for the development of parasites in temperate regions and, as a result, a modification is observed in the level and time of infection manifestation.

Based on the results cited above, the author and the scientific director conclude that the spread of gastrointestinal nematodes and the frequency of cases with reduced or missing effectiveness of the known deworming agents require a reevaluation of the current strategies for dealing with this type of infection and the development of new methods of limitation of economic losses in sheep farming. One of the most promising and long-lasting approaches is the LOW FEC trait team, to establish breeds or form populations with natural resistance. Another possibility is the establishment of animals that show tolerance to the parasites and accordingly their productivity is affected to a lesser extent.

**Conclusions.** The conclusions are 19 in number and reflect the obtained results in a concentrated form.

**Contributions.** A total of 13 contributions are presented, of which 5 are original and the rest are confirmatory.

**Practice recommendations.** Because the research carried out has not only a scientific focus but is also interesting for breeding and veterinary medical practice, the author offers 8 specific recommendations, which, if eventually implemented in practice, would lead to greater success in the fight against parasites in sheep. but would also limit the use of antiparasitic drugs, which in turn would reduce parasite resistance to drugs.

Based on the obtained results, the candidate has published three scientific articles:

1. Marincheva V. (2021) Selective criteria for resistance to gastrointestinal nematodes in sheep. Zhivotnovadni Nauki 58(4): 64-75.

2. Marincheva V., Kurtenkov A. (2022). Body condition scoring and body measurements of ewes with gastrointestinal nematode infection from Bulgaria. Zhivotnovadni Nauki 59(4): 10-15.
3. Marincheva V., Kanchev K., Manev I. (2023) Fecal Egg Count and FAMACHA Score in Ewes. Global Journal of Animal Scientific Research, 11(1), 1-15.

**CONCLUSION**

I found that in this thesis project, very detailed work has been done on the distribution, parasitization capabilities and natural resistance of the sheep species as a whole to gastrointestinal nematodes. Many factors influencing this complex biological process of host and parasite survival have been investigated. A lot of work has been done, which is full of content, and I believe that the results obtained will be useful not only for science, but also for selection work in sheep breeding. On the basis of these findings, I recommend to the honorable members of the Scientific Jury to award Assistant Professor Victoria Emilova Marincheva the educational and scientific degree "DOCTOR".

**REVIEWER:**

Prof. Dr. L. Sotirov