



OPINION

From: Professor Darinka Nedelcheva Ilieva, DVM, PhD, Scientific Specialty "Epizootology, Infectious Diseases and Prevention of Infectious Animal Diseases", National Diagnostic Research Veterinary Medical Institute, Sofia. Member of the Scientific Jury with Order № ZPS-170/01.04.11.2024 of the Rector of the University of Forestry, Sofia, Bulgaria.

Concerning: Dissertation on the topic: "**Impact of dietary supplementation with different herb species on body performance meat quality and health status in broiler chickens**", author Roger Hanna Al Hanna, the PhD student in extramural training at the Department "Anatomy, Physiology and Animal Science", enrolled by Order №77/26.02.2019 of the Rector of University of Forestry, Sofia, Faculty of Veterinary Medicine, for the acquisition of Educational and Scientific Degree "Doctor" (PhD), Field of higher education 6. Agricultural Sciences and Veterinary Medicine, Professional direction 6.4. Veterinary Medicine, Scientific Specialty "Animal Pathology".

Scientific supervisor: Assoc. Prof. Krasimira Genova, DVM, PhD at the University of Forestry-Sofia, Scientific Specialty "Animal Pathology"

Scientific consultant: Prof. Boulos Al Jammal, PhD at the Lebanese University, Faculty of Agronomy and Veterinary Medicine.

1. Relevance of the developed problem.

Nowadays people are at the limit of its ability to produce generics, which are effective for living organisms and at the same time, after treating them, do not build antimicrobial resistance. Antibiotic resistance is also a food safety problem: antibiotic use in the feed of the animals for treatment, disease prevention or growth promotion, allows the spread of resistant bacteria and genes coding antimicrobial resistance. It is known, that people die from infections which have entered in their organism with the food, caused by antibiotic-resistant bacteria.

In this sense the use of herbs and spices in poultry farming is a good alternative to conventional antibiotics. Herbs or botanicals in animals may arise feed intake and digestive secretion. The herbs have antibacterial, antiviral, antifungal, antihelmintic, coccidiostatic, antiinflammatory activity and antioxidant properties, stimulated the immune system, have a beneficial effect on the homeostasis of the organism, improve growth performance and quality of animal products. In this aspect the dissertation work is a modern scientific elaboration.

2. Structure and content of the presented dissertation.

The dissertation is presented on 153 pages of text, 10 tables and 30 figures. The literature index contains 408 author's titles in Latin, aranged in alphabetical order. The dissertation is structured according to the scheme; Title page-1page; Acknowledgments-1page; Abstract-1page; Contents-4pages; Abbreviations and Introduction-6 pages; Literature Review-37 pages; Purpose and Tasks-1 page; Materials and Methods-13 pages; Results and Discussion-49 pages; Conclusion and Recommendations-3 pages; Work Contributions-1 page; References-35 pages; List of publications related to the dissertation-1 page.

The dissertation developed by Hanna Al Hanna is according to the requirements for a Scientific specialty "Animal pathology". There is a balance in the volume of each of the sections, that include the necessary elements for dissertation. The literature review presents data on the poultry sector status in the Middle East, in the world and in Lebanon, management of the broiler flocks (feed and feeding system and rearing methods). There are data for

antibiotics use in poultry sector and effect of antibiotics on human health; phytogetic feed additives (herbs and spices), effects of using herbal feed additives; source of antioxidants and mode of action aromatic plants and essential oils and antioxidant plants important for the poultry health and for the oxidative stability of their products (meat). Phytobiotics play a growing role as potential alternative to antibiotic growth promoters (AGP) because they are natural, easily available, nontoxic and residue-free. Hematological and immunological data on broilers resulting from the use of herbs showing a good blood profile are also discussed. The birds' immune system and gut microbiota in vertebrates enhance the activity of natural killer cells (NK), granulocytes, macrophages, and cytokine response. The literature review is well formed and shows that the doctoral student is well acquainted with the scientific publications in the world literature, shows excellent knowledge and competence.

In the section "Goal and tasks", the goal is clearly formulated. The doctoral student evaluated in the dissertation work the impact of six local natural herbs and spices with the main goal the substitution of antibiotics conventionally used in the Lebanese broiler industry. For its implementation the following tasks are set: conducting a first trial where 8 groups of broiler chicken are fed by rosemary, chamomile, peppermint and thyme as supplements to the basal diets to evaluate their effects on health status, live body weight, feeding and feed conversion ratio, live body weight gain, which play an important role in the optimum growth of the birds, carcass yield, weight of internal organs and meat quality; conducting a second trial in which 5 groups of broiler chickens are fed by garlic and onion powders as supplements to the basal diets to evaluate their effects on the same indicators cited above; assessing the blood parameters, serum biochemistry profile and immunology; evaluating and comparing obtained results of both trials and drawing conclusions on potential alternatives for antibiotics-based on physiological status, immune response and quality of poultry meat.

The "Materials and Methods" section covers the equipment, experimental setup, site, distribution, conducted trials, studied indicators used serological tests and kits, as well as experimental broiler chicks. According vaccination program all birds are vaccinated against Gumboro disease, Infectious bronchitis and Newcastle disease. The PhD student conducted two trials consisting of 13 groups fed on 11 diets. Both trials conducted for 31 days in a poultry farm. Feeding of natural herbs and spices started after 20 days. Control groups (negative controls of trial I and trial II) were fed a basal diet without antibiotics and antioxidants. Control groups (positive controls of trial I and trial II) were fed basal diet with antibiotics (15 mg of virginiamycin/kg and antioxidant 125 mg/kg, ethoxyquin). The average values of each group (trial I and trial II) were statistically calculated. During both trials, the birds are given *ad libitum* access to feed and water. In both trials, three-phase feeding of birds was used, as well as rearing them under the same environmental conditions. The chicks are fed with commercial broiler starter diet from day 1 to 19 and a commercial broiler grower diet from 20 days until slaughter 31 days, according the manufacturer with vitamin premix and mineral premix are provided to basic diet (per kg of diet): All diets fed are in the form of mash, formulated and prepared commercially to be isocaloric and isoproteinic. The all fed diets are formulated to meet minimum nutrient requirements by the National Research Council (NRC, 1994).

In the dissertation work the doctoral student investigated-health status and mortality; live body weight (LBW) and feed intake (FI) were determined at the end of each feeding period (19 and 31 days of age) where feed conversion ratio (FCR) was calculated; live body weight gain (LBWG); carcass yield (%); weight of liver + heart; breasts (%) to net weight; meat quality measurements; postmortem breast muscle pH at 24 h and after 48 h using pH meter; meat colour at 24 h and after 48 h; lightness, redness, yellowness using colorimeter; water holding capacity and drip loss (%) was calculated as a percentage (%) relative to the initial muscle weight after 24 h and 48 h; thawing loss (%); cooking loss (%); meat

tenderness. Hematological indices and serum biochemistry; hemoglobin concentration (Hb), pack cell volume (PCV); red and white blood cells (WBCs) determined Neuber count chamber; antioxidant enzymes; the ferric reducing ability of plasma (FRAP) and lipid hydroperoxides with spectrophotometric assays. Catalase and levels of immunoglobulin (IgA, IgM and IgG), respectively malondialdehyde (MDA) by Elisa kit, Elabscience Biotechnology Co., Ltd. (Houston, TX, USA). Serum proteins (albumin and globulin), total cholesterol, creatinine: Beam spectrophotometer (492 nm); alanine transaminase (ALT), aspartate transaminase (AST) and low-density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides are determined of the procedures described by Burtis and Ashwood (1999). I believe, that the researched materials are sufficient and suitable for obtaining accurate results.

In the "Results and discussion" section, the data are presented in text, in tabular form and are demonstrated with figures. From this section it can be seen, that the author has a thorough knowledge of the biochemical and serological methods as ELISA used in the dissertation. The conducted experiments showed, that tested herbs have comparable effects to antibiotics on chicken growth and meat quality. In the dissertation, the doctoral student has completed the tasks set and satisfactory results have been achieved. The results were formed by statistical methods and showed that rosemary (1%) had a beneficial effect on broiler chicks growth. The herbs chamomile (1%), as well as thyme and mint mix (0.5% of each) improved the taste of feed. As for the blood parameters and serum biochemistry, the use of herbs has a noticeable impact in diets supplemented with a mixture of chamomile and rosemary where it increased pack volume cell (PVC), hemoglobin, white blood cells (WBC), eosinophils, total protein, albumin, alanine aminotransferase (ALT), high density lipoproteins (HDL) in the birds. Moreover low density lipoproteins (LDL) concentration decreased in the same groups, lowered lipid hydroperoxides (LOOH) and malondialdehyde (MDA), which is a clinical and laboratory marker of oxidative stress in organism and it is the most mutagenic product of lipid peroxidation. Rosemary supplementing in the diets increased heterophils, lymphocytes, monocytes and mean corpuscular hemoglobin, while chamomile usage increased globulin concentration. In both mixtures chamomile/rosemary and garlic/onion in the diets improve the average values IgG and IgM. The results in the dissertation showed use of natural herbs as growth promoters could decrease the reliance on antibiotics. Discussion of the results shows, that the PhD student has the ability to analyze, summarize and discuss the data obtained by comparison with the data of other researchers.

In the dissertation work are presented five original contributions both (scientific and practical), conclusion and recommendations, that I accept.

The dissertation presented to me by Roger Hanna Al Hanna "**Impact of dietary supplementation with different herb species on body performance meat quality and health status in broiler chickens**", has not plagiarism. The doctoral student has signed a declaration of authorship of the dissertation thesis for the acquisition of Educational and Scientific Degree (ESD) "Doctor" (PhD). There are no numerical indicators presented by the PhD student - citations, IF, etc.

Roger Hanna Al Hanna has 3 scientific articles related to the dissertation. The articles, published in the period (2018-2022). Two of them co-authored with other scientists in IX International Scientific Agriculture Symposium "AGROSYM 2018", BIH, published in Book of Proceedings, as well as one article is independent in Journal of World's Poultry Research whit 2 year Impact Factor/IF (2022-2023)=0,186; Cite score/CS (2021-2022)=0,6.

From the Reference for the implementation of the Minimum National Requirements by groups of indicators for acquiring of the Educational and Scientific Degree "Doctor", the doctoral student has the minimum required points from scientific publications.

Abstract is an integral part of the dissertation and reflects the sequence of the conducted researches, results achieved, work contributions, conclusions and recommendations for the practice.

3. Notes in connection with the dissertation.

I recommend, that the work on the scientific topic be continued and deepened with researchers from the country and abroad and the results achieved to be reported to appropriate scientific forums. To make popular the results so, that they are accessible to the specialists in practice.

CONCLUSION:

The dissertation is a modern scientific development with practical work contributions. The tasks set are fulfilled, as a result of which the goal is achieved. The doctoral student has proven that the use of natural herbs and spices had favorably effect in the health status and quality of meat and can decrease the reliance on antibiotics in basic broiler diets. Herbs (chamomile, rosemary, thyme, peppermint, garlic, onion) improved the palatability for feed, feed conversion, stimulated body growth, ameliorated immunity, as well as improved the blood parameters and serum biochemistry with a mixture of rosemary and chamomile.

The doctoral student has fulfilled the minimal national requirements (MNR) by groups of indicators, according to the Law on the Development of Academic Staff of the Republic of Bulgaria, Regulations for its implementation and Internal Rules for Development of the Academic Staff of the University of Forestry, Sofia. This gives me reason to evaluate positively the dissertation on "**Impact of dietary supplementation with different herb species on body performance meat quality and health status in broiler chickens**" and to recommend to the esteemed scientific jury to award Roger Hanna Al Hanna of educational and scientific degree "Doctor of Philosophy", field of higher education 6. Agricultural Sciences and Veterinary Medicine, professional area 6.4. Veterinary Medicine, scientific specialty "Animal pathology".

Sofia, 2024

Member of the Scientific Jury:
(Prof. Darinka Ilieva, DVM, PhD)