

LAVENDER ESSENTIAL OIL AS A FACTOR IN REDUCING THE EMISSION OF PATHOGENIC MICROORGANISMS THROUGH THE FAECES OF BROILER CHICKENS

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Abstract. Forty Ross 308 hybrid broiler chickens at the age of 20 days were included in the study. They were raised under the same conditions in the vivarium of the FVM at LTU - Sofia in bird cages with 10 chicks per cage. Twenty of the chickens (experimental group) received *Lavandula angustifolia* Mill essential oil in the daily feed ration at a final concentration of 0.01% or an average of 5 mg per chicken, from the 20th day to the 42th day of age. Pooled faecal samples were taken from all birds in both groups on the first day, on the 15th day after their arrival (in the middle of the experimental period) and at the end of the experiment. Microbiological studies of the samples were carried out, as for the isolation of microorganisms cultures were made in elective and selective nutrient media for bacteria from different groups, as well as for fungi, and their identification was carried out biochemically with the help of polymicrotests. Intake of lavender essential oil with food resulted in a more than two-fold reduction in the number of bacterial species in their faeces from major groups including pathogenic species as well as *Candida ovale* fungi. These data show that lavender oil exerts a significant antimicrobial effect *in vivo*, as a result of which their feces contain significantly less microorganisms with pathogenic potential and, accordingly, they are much lower dangerous from an epizootological point of view than those of normally fed birds.

Key words: broiler chickens, lavender essential oil, feces, pathogenic microorganisms

INTRODUCTION

Poultry farming is a rapidly growing industry, supported by huge customer demand. Data from the Ministry of Agriculture and Food (2024) indicate that consumption of chicken meat and eggs in the last months of 2023 in our country amounted to 90 500 tons, without significant change compared to previous years, and in the last two months of 2023 there was an increase in hatching eggs for broilers (by 7.2% on an annual basis) and in the sale of one-day-old broilers (by 5.7%), as a result of which it can be expected that the industrial production of chicken meat in early 2024 will be slightly above the level of a year ago.

The huge amount of manure litter generated by this production is a major environmental problem, and its recycling is lengthy and associated with a number of difficulties (Popova et al. 2007; Popova et al., 2009; Popova and Baykov, 2012; Popova et al., 2013). The deposition of raw manure for organic fertilization in the soil without prior treatment leads to adverse environmental consequences, as it often contains pathogenic microorganisms and parasite eggs that spread through it. In addition, it is a favorable environment for the reproduction of pathogens, attracts flies, pests, and the odor emitted is also a problem. Treatment with chemicals poses many risks of harm to the environment, animals and insects, and subsequently - to humans (Manogaran et al., 2022).

A number of authors have established a positive effect of the aromatic herb lavender on the body

weight of broiler chickens and quails, and a decrease in the number of microorganisms in their intestinal microflora (Adaszyńska-Skwirzyńska et al., 2019; Naderi et al., 2021; Amer et al., 2022; Özbilgin et al., 2023). Lavender is an easily recognizable evergreen plant with purple-blue aromatic flowers from the genus *Lavandula*, family Lamiaceae. Its flowers have been used to treat various diseases since ancient times (Kajjari et al., 2022). The essential oil obtained from it is highly valued in many and diverse fields such as the medical, perfumery, cosmetic, and food industries, due to its valuable and rich content of more than a hundred components, including: linalyl acetate, linalool, terpinen-4-ol, lavandulol, lavandulyl acetate, 1,8-cineole, limonene, cis- and trans- β -ocimene, and others (Pokajewicz, 2021). Thanks to these components, lavender essential oil has numerous beneficial properties and exerts antimicrobial, antioxidant, sedative, and antidepressant effects (Konluvad et al, 2013).

The data on the beneficial stimulating effect of lavender essential oil on the development of chickens and its proven antimicrobial properties determined the direction of our studies. The aim of the present work is to investigate the possibility of using lavender essential oil to reduce the microorganisms emitted through the feces of broiler chickens from groups containing important pathogenic species and thus to reduce the dangers of their spread through the manure litter in the environment.



MATERIALS AND METHODS

Essential oil. Lavender essential oil was used – *Lavandula angustifolia* Mill., varieties Sevtopolis, Hemus and Yubileyna (Fig. 1.), produced in Bulgaria by

the company "Bioagro Bg OOD". The chemical composition of the essential oil was determined using the Lavender_DB-WAX_Test.M method at Pavlovi Food Industries Laboratory, and certificates were provided.



Fig. 1. *L. angustifolia* Mill. from which the essential oil was produced in flowering phase (orig.)

Chickens. We used 40 twenty-day-old broiler chickens of both sexes, Ross 308 hybrids, purchased from a private farm with registration number 1247-0079. They were raised in poultry cages with 10 chickens per cage (Fig. 2) in compliance with all

requirements for animal hygiene and animal welfare (Permit for the use of animals in experiments No. 277/02.07. 2020, valid until 20.07.2025, Institute of Animal Sciences, Kostinbrod).



Fig. 2. A part of the chickens used in the experiments.

Food. Throughout the entire period of the study, the birds were fed with complete granular feed “FARM 2 pellet” from the Belgian manufacturer of feed, food, supplements, accessories and 9* other products for animals “Versele-laga”, purchased from “Bora Vet OOD” and “Gergovche”, as well as from the company “SAM BS”, Sofia. According to the manufacturer’s instructions, the feed is suitable for broiler chickens from 11 days of age until 5 days before slaughter in an amount of 50 to 150 g per chicken per day. It contains wheat, genetically modified soybeans, corn, rapeseed, products from the bakery and pastry industry, palm kernel expeller, calcium carbonate, beet molasses, monocalcium phosphate, sunflower oil, sodium chloride and sodium bicarbonate. The diet of the chickens was without additional feed additives, vitamins or antibiotics. During the first and second weeks of the experiment, the ration (dry feed) of each group was 1 kg twice daily.

Experimental setup. After five days of adaptation to the new food and environment, the 25-day-old chicks were divided into two groups (group A-experimental and group B-control) with twenty individuals in each, with an equal number of male and female birds. For 30 days, the diet of group A was supplemented with lavender essential oil (*Lavandula angustifolia* Mill), produced in Bulgaria in 2022 and 2023 by Bioagro Bg Ltd, with the relevant quality and composition certificates. During the first two weeks of the start of the experiment, we applied a total of 113 ml of lavender essential oil (0.885 mg/ml), which we dissolved in 100 ml of 80% ethanol, then added water to 1000 ml. Each 1 ml of this solution contains 100 µg of pure essential oil. We offered the feed by adding drinking water to it until it was completely wetted in the form of a slurry, and then adding the diluted lavender oil and mixing thoroughly. In this way of preparation, we achieved a more even distribution of lavender oil in the daily ration, in a final concentration of 0.01% or an average of 5 mg per chicken. Adding water to the feed increased its volume and so its total amount at each feeding was 1,500-1,600 kg per 10 birds. In a similar way, we prepared the diet of individuals from the control group B by wetting the same amount of feed until it was obtained as a slurry, but without adding lavender oil to it. The chickens were fed with the indicated portions twice a day, and water was provided *ad libitum*.

On the third week of the experiment, due to the intensive increase in body weight of the animals, it was necessary to adjust and provide 0.200 kg more dry feed per 10 birds. Accordingly, we increased the amount of lavender oil added to group A by 118 ml

twice daily to achieve the same final concentration of 0.01%.

Material for microbiological studies. At the end of the experiment, pooled fecal samples were taken from the birds of the experimental and control groups.

Microbiological studies. For the isolation of microorganisms, cultures were made in elective and selective nutrient media for bacteria from different groups, as well as for fungi. They were cultivated at 37 °C and 28 °C for 24 – 72 h under aerobic conditions.

Nutrient media. For the isolation of microorganisms from the studied groups and species, elective and selective media (Merck-Bio Lab, Bulgaria) were used. Eosin Methylene Blue agar for *E. coli* and Gram-negative aerobic bacteria, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus *Staphylococcus* and Sabouraud agar for fungi were selected.

The taxonomic identification of the isolated bacteria was performed by conventional methods according to the 9th edition of the Bergey's Determinant (Holt et al., 1994). It was carried out by taking into account their cultural properties on nutrient media and biochemical characteristics using tests for biochemical identification of staphylococci, enterobacteria and fungi (HiMeida Laboratories Pvt. Ltd. Mumbai India). According to the instructions of the tests, suspensions were prepared in physiological solution with a certain density and 0.1 ml were dropped into the corresponding wells of the test plates. The results were read after incubation at 37°C for 24 h. The determination of the species affiliation of the isolated microorganisms was carried out according to the color keys attached to the tests, determination of the digital codes of the isolated bacteria and their finding in the code books.

The isolated Gram-negative aerobic and facultative anaerobic bacteria were identified using the MICROLATEST ENTERO test 24 N. We prepared suspensions in saline from 24-hour bacterial cultures of microorganisms isolated from chicken fecal samples on Eosin Methylene Blue agar. We homogenized the suspensions well with a Vortex and brought them to a turbidity of 1 according to the McFarland standard. We inoculated 0.1 ml into all wells of the plate. In the first five wells, we placed 2 drops of sterile paraffin oil, since the strong alkaline reactions of some tests (for decarboxylase, indole, urease, hydrogen sulfide, etc.) can affect the test results in the remaining wells (Fig. 1). After recording the reactions for indole, urease, hydrogen sulfide, etc., we placed the plate in an incubator at 37 °C for 24 hours. We recorded the results using the color index provided with the test and recorded the data (Fig. 2). We performed the recording

and interpretation of the results using the test codebook.

The speciation of staphylococci isolated from the fecal samples of broiler chickens on Chapman Stone agar was carried out using MICROLATEST STAPHYtest 24, which is designed to identify microorganisms of the genus *Staphylococcus* and related Gram-positive catalase-positive cocci. We worked with 24-hour cultures, from which we prepared suspensions in saline with a density of 2 according to McFarland. We homogenized them well on a Vortex and inoculated 0.1 microliters into all wells of the plates. We placed 2 drops of sterile paraffin oil in wells H, G and F of the first row. We incubated the prepared plates at 37 °C. for 24 hours. We read the results according to the color code for the test, and after determining the eight-digit codes of the strains, we found the corresponding species in the code book for the test.

To identify the isolated Sabouraud agar tubes from the fecal samples of broiler chickens, we used MICROLATEST CANDIDA-Screen, designed for the identification of the most common clinically significant species of oval fungi. According to the instructions for performing and reporting the test, we

prepared suspensions in saline from 24-hour cultures with a turbidity of 3 McFarland, which we homogenized well on a Vortex. We inoculated 100 microliters into the wells of the plate, then placed 3-4 drops of sterile paraffin oil in all inoculated wells. We placed the prepared plates in a thermostat for 24 hours. We read the results by comparing the color reactions with the color index and obtained the eight-digit codes of the strains, which we identified using the code index of the test.

RESULTS

The isolated Gram-negative bacteria from the examined fecal samples of the experimental (fed lavender essential oil with food) and control (fed and raised in the same way, but without the addition of essential oil) broiler chickens are presented in a comparative aspect in Table 1.

The significant species superiority of Gram-negative bacteria with pathogenic potential in the feces of the control chickens that did not receive essential oil is striking. Five species were isolated from them, and from the chickens fed with the addition of lavender oil – only two species of conditionally pathogenic Gram-negative bacteria

Table 1. Gram-negative bacteria isolated from the examined fecal samples of the experimental and control broiler chickens.

Species of isolated Gram-negative bacteria	
Experimental group (received lavender oil)	Control group
<i>K. pneumoniae ssp. ozaenae</i>	<i>K. pneumoniae ssp. ozaenae</i>
<i>Serratia marcescens</i> biovar 1	<i>Serratia fonticola</i>
	<i>Proteus vulgaris</i>
	<i>Citrobacter freundii</i>
	<i>Pseudomonas fluorescens</i>

The results of the studies of Gram-positive cocci in the feces of the experimental and control groups of chickens are similar, which can be seen in Table 2.

Some results of the biochemical identification of the isolated staphylococcal species can be seen in Fig. 3.

Table 2. Gram-positive cocci isolated from the examined fecal samples of the experimental and control broiler chickens.

Species of isolated cocci	
Experimental group (received lavender oi)	Control group
<i>Staphylococcus piscifermentans</i>	<i>Staphylococcus piscifermentans</i>
<i>Aerococcus viridans</i>	<i>S. warneri</i>
	<i>S. simulans</i>
	<i>Staphylococcus lutrae</i>

Only two types of Gram-positive cocci were isolated from the feces of the chickens that received

lavender essential oil, half as many as the types isolated from the control birds.

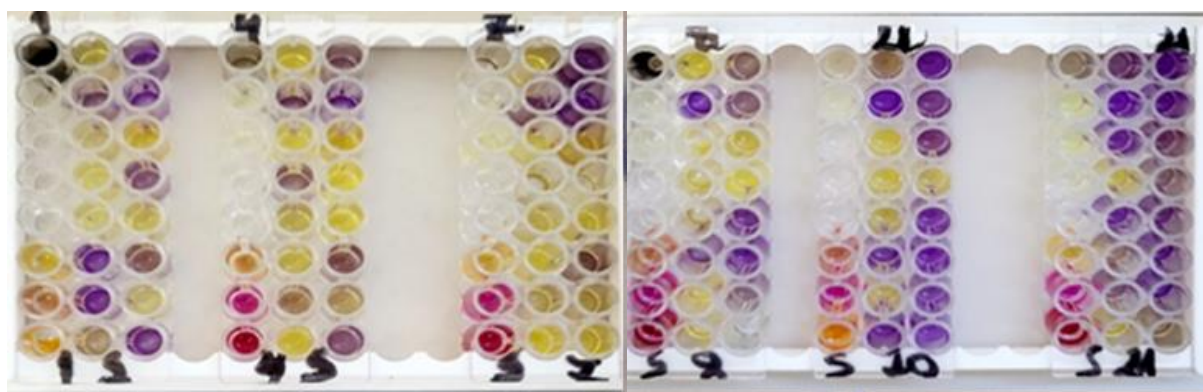


Fig. 3. Results of the biochemical identification of some of the isolated staphylococcal species.

Table 3 presents the results of the studies for the presence of fungi in the fecal samples of the broiler chickens.

Table 3. Oval fungi isolated from the examined fecal samples of the experimental and control broiler chickens.

Types of isolated fungus	
Experimental group (received lavender oi)	Control group
<i>Geotrichum sp.</i>	<i>Geotrichum sp.</i>
	<i>Candida krusei</i>
	<i>Trichosporon sp.</i>

As can be seen from the data in the table, only a saprophytic fungus of the genus *Geotrichum* was isolated from the chickens that received lavender oil with food, which was also present in the feces of the control individuals. However, in the chickens that did not receive the essential oil, there were fungi of two more species that were conditionally pathogenic.

DISCUSSION

Our results are in line with those of Adaszyńska-Skwirzyńska et al. (2019), who reported that the addition of natural lavender essential oil to the drinking water of chickens had a positive effect on the intestinal microflora of the ileum, with the amount of pathogenic microorganisms decreasing (*E. coli* and coliforms), while the number of probiotic bacteria significantly increasing. Özbilgin et al. (2023) found that lavender essential oil added to quail diets led to an increase in the number of probiotic bacteria from *Lactobacillus* spp. Naderi et al. (2021) also reported that the dietary administration of lavender essential oil and antibiotics from the bacitracin methylene disalicylate group to Japanese quails had a beneficial effect on the intestinal microflora, resulting in a decrease in the amount of *E. coli* and an increase in *Lactobacillus* sp. Furthermore, linalool and linalyl acetate, which are contained in this oil, protect against acute gastric ulcers induced by ethanol, but do not prevent indomethacin-induced lesions, indicating a lack of interference with the arachidonic acid metabolic cascade. Analgesic activity with gastroprotection was also found after inhalation of lavender essential oil (Barocelli et al., 2004).

Baker et al. (2012) demonstrated that colitis in mice caused by *Citrobacter rodentium* was inhibited by lavender oil. The application of this essential oil suppressed the signs of inflammatory bowel disease, including Crohn's disease and ulcerative colitis. It resulted in reduced morbidity and mortality, reduced intestinal tissue damage, and reduced infiltration with neutrophils and macrophages, reduced levels of TNF- α , IFN- γ , IL-22, and other positive effects. This was associated with increased levels of regulatory T cell populations compared to untreated mice with colitis. At the same time, it maintained the intestinal microbiota with a specific increase in bacteria from the Firmicutes division and a decrease in γ -Proteobacteria (Baker J. et al., 2012). Lavender essential oil is able to regulate the level of inflammatory factors, inhibit inflammatory reactions through multicomponent and multitargeted action, and ultimately alleviate the damage to the colonic mucosa of mice with acute colitis (Wang et

al., 2021). Furthermore, it was experimentally found that the aqueous extract of *Lavandula stoechas* L. exhibited significant antiulcer activity in ethanol-induced gastric ($P < 0.001$) and duodenal ($P < 0.01$) ulcers in rats compared to controls. The aqueous and ethanol extracts of *L. stoechas* L. showed strong free radical scavenging activity (Javed, 2020).

An interesting recent study from June 2024 demonstrated that simply inhaling lavender essential oil altered the gut microbiota composition of alcohol-abstinent rats, increasing the abundance of species from the Muribaculaceae family. Improvements in their gut metabolites were also observed. Transcriptomic analysis used in this study revealed that lavender essential oil regulated inflammatory factors and gene expression in the hippocampus, inhibiting the inflammation-related gene *Trpv4* (Li et al., 2024).

The Gram-negative bacteria isolated by us from the feces of the studied birds belong to the *Enterobacteriaceae* family, with the exception of *Pseudomonas fluorescens*, which belongs to the *Pseudomonadaceae* family. No obligate pathogenic species were identified. *K. pneumoniae* ssp. *ozaenae* isolated from both groups of chickens rarely causes serious diseases in animals and humans, but may be involved in the development of atrophic rhinitis in pigs. It has not been reported to cause pneumonia in humans (Miacevic et al., 2022). This also applies to *Serratia marcescens*, which was initially considered non-pathogenic due to its low virulence in healthy populations. However, over the past 30 years, its importance has increased and it has been isolated in various infectious diseases, including urinary, respiratory and biliary tract, peritonitis, complicated wounds and intravenous catheter-related infections, which can lead to bacteremia. Risk factors include prolonged antimicrobial or immunosuppressive therapy, and in humans, frequent catheterization and underlying diseases such as chronic lung disease and diabetes mellitus (Kim et al., 2015). *Serratia fonticola*, which we isolated from the control group of broiler chickens, is widespread in the environment, including drinking water, soil and sewage. It is a possible pathogen and has been isolated in paraplegia and urosepsis (Aliorayid et al. 2016).

P. vulgaris, which we isolated from the feces of the control group of broiler chickens, is conditionally pathogenic for animals and humans. Its virulence factors contribute to reaching different parts of the host body and the possibility of developing infections, mainly in patients with a damaged immune system. It can complicate urinary tract infections and wounds, as well as cause nosocomial

infections. *Proteus* spp. are common but undesirable representatives of the intestinal microflora, as they can become causative agents of diarrhea and other diseases of the digestive system (Drzewieska, 2016). *Citrobacter freundii*, which we also isolated from the control group of broiler chickens, under unfavorable circumstances for patients can cause urinary or respiratory tract infections, meningitis and sepsis (Wander et al., 2017).

We isolated only two species of saprophytic Gram-positive cocci from the feces of chickens fed lavender essential oil. However, fecal samples from the control group contained twice as many staphylococcal species, two of which (*S. warneri* and *S. simulans*) were opportunistic. *Staphylococcus piscifermentans* is a non-pathogenic species isolated from fish, shrimp, and Malaysian traditional fermented shrimp as a lactic acid fermentation bacterial strain, but Onyilokwu et al. (2021) also isolated it from the nasal mucosa of healthy ruminants and their keepers in Nigeria. *Aerococcus viridans*, which we identified in the feces of the experimental group of chickens, is a Gram-positive, catalase and oxidase-negative, microaerophilic, non-motile cocci that are very rarely isolated in human infections and are usually considered contaminants (Ezechukwu et al., 2019).

The genus *Geotrichum*, whose representatives we identified in the feces of chickens from both study groups, includes 18 species of ubiquitous filamentous yeast-like fungi, commonly isolated from soil, air, water, milk, silage, plant tissues, and the digestive tract of humans and other mammals. *Geotrichum candidum* is widely used as an additional culture in cheese ripening. Fungi of the genus *Geotrichum* are pathogens of some plants, causing acid rot of fruits and vegetables (Pottier et al., 2008; Thomidis et al., 2021).

Candida krusei, isolated by us from the feces of control chickens, is a diploid, dimorphic ascomycete yeast-like fungus that is found on the mucous membranes of healthy individuals. However, it can cause life-threatening infections in immunocompromised patients, such as those with hematological malignancies, as well as those using long-term azole prophylaxis or therapy (Jamiu et al., 2021). *Trichosporon* spp., which we also isolated from the feces of control birds not receiving lavender oil, are basidiomycete yeast-like fungi that are widely found in nature. Clinical isolates are usually associated with superficial infections. Recently, these fungi have been recognized

as opportunistic agents of invasive infections, especially in cancer patients and those exposed to invasive medical procedures (Colombo et al., 2011).

The significant reduction in the number of Gram-negative and Gram-positive microorganisms in the feces of chickens fed lavender essential oil compared to control birds is clearly due to the antimicrobial effect of the oil. It has been conclusively demonstrated *in vitro* against microorganisms from the groups we identified, including oval fungi, both in our previous studies (Mladenova et al., 2024) and by other authors (Said et al., 2015; Poole et al., 2022; Tkachenko et al., 2022; Truong and Mudgil, 2023; Mourabiti et al., 2024).

CONCLUSIONS

A significant species predominance of Gram-negative bacteria with pathogenic potential was found in the feces of control chickens that did not receive essential oil. Five species of opportunistic Gram-negative bacteria were isolated from them, and only two types of conditionally pathogenic Gram-negative bacteria were isolated from the chickens fed with the addition of lavender oil. Only two types of Gram-positive cocci, which are saprophytic, were isolated from the feces of the chickens receiving lavender essential oil. In the fecal samples of those from the control group, however, twice as many staphylococcal species were found, two of which (*S. warneri* and *S. simulans*) were opportunistic. Only a saprophytic fungus of the genus *Geotrichum* was isolated from the chickens fed lavender oil, which was also present in the feces of the control individuals. However, in the chickens that did not receive the essential oil, there are two more species of fungi that are conditionally pathogenic.

The essential oil of *Lavandula angustifolia* Mill, applied daily in the daily feed ration of broiler chickens at a final concentration of 0.01% for 21 days, exhibits antimicrobial activity *in vivo*, as a result of which their feces contain more than two times fewer species of Gram-negative bacteria, staphylococci and oval fungi with pathogenic potential compared to those fed without the addition of essential oil. Their feces are much less dangerous for the environment as a source of pathogenic microorganisms compared to those of conventionally fed birds.

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