DOCTORAL DISSERTATION SUMMARY

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DEVELOPMENT AND EVALUATION OF ANTIMICROBIAL EFFICACY OF A MIXTURE OF NATURAL ESSENTIAL OILS FOR PROPHYLACTIC FOGGING OF TURKEY HOUSES

The intensive poultry housing system and the specific microclimate formed in farm buildings contribute to the accumulation of bioaerosol in the birds' living environment. Its main components are bacteria and fungi, including pathogenic or opportunistic ones, as well as their metabolites. The combined effect of biological agents and other air pollutants is considered one of the most important stress factors in bird rearing. Harmful environmental factors negatively affect the structure and defence functions of mucous membranes, especially of the respiratory system and conjunctiva, causing allergic reactions, inflammation and increased incidence of infectious diseases in birds. In flocks of slaughter turkeys, diseases of complex bacterial aetiology that require treatment, including antibiotics, are frequently observed. In turn, the widespread use of antimicrobials in the treatment of bacterial infections on poultry farms leads to increasing antibiotic resistance of bacterial strains. Therefore, in the search for effective methods to reduce contamination levels in poultry production, natural methods are gaining interest. Due to their rich chemical composition and wide range of biological activity, essential oils have great potential in this respect. All available scientific publications in the field of antimicrobial efficacy of essential oils present results of *in vitro* tests, mainly on commercial standard strains, and their application in poultry production is limited to their use in nutrition. Preliminary studies conducted by the research team of Witkowska et al. (2013, 2016, 2019) showed that the use of aqueous solutions of single essential oils in aerosol form reduces microbial contamination in the environment of broiler chickens. However, as highlighted by the aforementioned authors, it is important to remember that not all oils exhibit antimicrobial properties and those that do may not be effective at low concentrations. Some essential oils available on the market, despite many beneficial properties, may be toxic to animal organisms, therefore, further research is necessary for their proper selection and application. To address these issues, in this study, sensitivity tests were performed on previously isolated microorganisms from turkey farms.

Therefore, the aim of this work was to determine the most frequently occurring microorganisms and drug resistance of pathogens on turkey fattening farms, then to develop a natural preparation based on selected essential oils for prophylactic fogging of farm buildings intended for raising turkeys and to test the antimicrobial efficacy of a natural multicomponent preparation based on essential oils after application in the form of fogging in the living environment of farm turkeys.

The study was conducted in three stages. The material for the first stage of the study comprised the results of bacteriological analyses of internal organs of turkeys kept on farms located in north-eastern Poland, stored in the archives of the Department of Veterinary Hygiene in Olsztyn. This stage of research showed the most frequent occurrence in flocks of slaughter turkeys of bacterial strains such as: *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Ornithobacterium rhinotracheale*. The second stage of the study was carried out in the laboratory and comprised the development of a natural oil preparation with antimicrobial properties for prophylactic fogging of air in buildings for slaughter turkeys. Field isolates of bacteria and twelve 100% natural essential oils were used for this purpose. The most effective essential oils and their minimum inhibitory concentrations (MIC) were determined using the disk-diffusion, well and microdilution methods. Based on the results, individual essential oils were selected and mixed with appropriate emulsifiers and distilled water. The obtained preparation was tested for its effectiveness using the *in vitro* method. In the third stage of the study, carried out on a turkey fattening farm located in the north-east of Poland, the antimicrobial efficacy of the natural broad-spectrum essential oil preparation was evaluated in the turkey rearing environment. For this purpose, air and litter samples as well as swabs from walls, drinkers and feeders were taken. At this stage of field and laboratory studies, the microbiological analysis included quantitative and qualitative identification of environmental microflora.

Based on the results of the study, the following generalisations and conclusions can be made:

1. No seasonality is found in the occurrence of *Escherichia coli*, *Klebsiella pneumoniae*, *Ornithobacterium rhinotracheale*, *Staphylococcus aureus* and *Enterococcus faecalis* bacteria on slaughter turkey farms.

2. In the internal organs of turkeys for fattening, *Enterococcus faecalis* bacteria (including MDR strains) and *E.coli* are identified most frequently, and *Ornithobacterium rhinotracheale* bacteria are identified least frequently.
3. The variability of the antibacterial activity of individual essential oils is determined by the type of bacterial strain tested, the concentration of the oil and the test method used.
4. Cinnamon, clove and thyme oils show the highest antibacterial activity against both G+ and G- microorganisms. Lemon oil shows very weak activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Ornithobacterium rhinotracheale*, *Staphylococcus aureus* and *Enterococcus faecalis* from slaughter turkeys.
5. The application of the essential oil-based preparation by fogging reduces the occurrence of microorganisms in the rearing environment of turkeys for fattening.
6. The use of a number of selected essential oils within one preparation enhances the effects and increases the antimicrobial activity of the mixture.
7. Air fogging with multicomponent preparation based on essential oils shows high potential of the applied mixture in maintaining hygienic standards on turkey farms and may prove effective in preventing pathogenic (including multiresistant) bacterial isolates originating from turkey farms.
8. Further studies aimed at improving the composition of the natural preparation based on essential oils and its effects on the organisms of birds are recommended.

Keywords: microorganisms, drug resistance, essential oils, antimicrobial properties, mixture of essential oils, turkey house, fogging, prophylaxis, microbial contaminations