THE RESEARCH OF LITTER IN POULTRY HOUSE AND USE OF ESSENTIAL OILS IN BROILER PRODUCTION

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Pроведено аналіз літературних джерел щодо актуальності вивчення та лабораторного дослідження підстилкових матеріалів у сучасному птахівництві. Виявлено, що підстилка є не тільки накопичувачем забруднюючих речовин, поживним середовищем для існування патогенних та умовно-патогенних мікро-організмів, але й може здійснювати збільшення емісії шкідливих газів, таких як аміак, вуглекислий газ та сірководень у разі порушення технології вирощування птиці. Таким чином завдаючи негативного впливу як птиці так і обслуговуючому персоналу птахопідприємств.

Мета. Здійснити хімічний та мікробіологічний аналіз підстилки та дослідити бактерицидні властивості ефірних олій.


Результати досліджень. На початковому етапі вирощування птиці за допомогою лабораторних досліджень було виявлено, що у досліджуваних зразках підстилки вологість складала 22,1 %, а кількість сухої речовини – 77,9 %. На завершальному етапі вирощування птиці в результаті мікробіологічного дослідження в один зразок виявлена присутність лактозопозитивних кишкових паличок інтенсивністю 3, що свідчить про її належний санітарний стан.

Висновки. У результаті хімічного аналізу підстилки показано наявність не тільки хімічних елементів, домішок (тирсу деревини та негашеного вапна), але і присутність органічної речовини у вигляді сирового жиру, сироваткових і білкових фракцій, які завдають негативного впливу на птицю у разі відсутності хімічних засобів обробки підстилки.

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**1. Introduction**

Modern production of poultry products provides the population with a high quality dietary product. However, the intensification and expansion of poultry production and increase of the size of poultry enterprises leads to harmful ecological effects [1].

According to [2, 3], the main problem that will concern livestock in future − is this ecological situation environment. The dynamic development of poultry has a negative impact on the environment, due to poor utilization of by-products of poultry farming, litter, emissions of significant volumes of exhales, dust, development of insect pest groups, unpleasant smell near poultry enterprises, etc. In addition, the intensive production of poultry products causes greenhouse gas emissions into the atmosphere, soil contamination and eutrophication of water bodies [4].

The impact of poultry farming on the state of the environment depends on a number of factors, including: size of the farm, production system, composition of the diet of the poultry, type of litter, etc. It is known that proper management of poultry wastes, especially poultry litter, can be a valuable resource, that is, it can be used as a fertilizer, soil feeder or as a source of energy [5]. Without proper treatment of poultry litter is an accumulator not only of pollutants, but also pathogenic or conditionally pathogenic microorganisms, which can get into the environment.

The search for environmentally safe ways of disinfection of poultry waste is a promising scientific direction. The research confirms the efficiency of the use of plant extracts that is in the future it will be possible to refuse chemical means of processing of poultry waste harmful to the environment [6].

In addition, essential oils of medicinal plants have immunocorrective properties, which helps to reduce the morbidity and increase the preservation and productivity of the poultry.

**2. Literature review**

Almost all poultry population with egg production technology is grown in battery cages and poultry used for meat production (parent poultry, repair young animals, ducks, geese, turkeys, as well as young poultry grown for meat) − mainly on the floor, on deep litter. Therefore, due to the rapid growth of poultry meat production, especially broiler meat, in recent years, the industry has an increasing demand and deficit of litter materials [7].

It is common knowledge that in the litter under the optimal conditions of the microclimate of the poultry house can develop such pathogenic microorganisms as Salmonella, Campylobacter, Coliform, Proteus, fungi of the genus Aspergillus, etc. Increased temperature and humidity, a sufficient amount of nutrients contribute to the rapid growth and development of microorganisms. It is strictly forbidden to use mold, frozen, wet and not checked by the laboratory for the presence of aspergillo-sis spores in litter. The humidity of the litter materials should be no more than 20 % according to the technology of poultry breeding [8, 9].

In addition to the accumulation of harmful metabolites and microorganisms, the litter has the ability to emit significant amounts of harmful gases − ammonia (NH₃), carbon dioxide (CO₂) and hydrogen sulfide (H₂S). According with the technological standards of designing poultry enterprises, the allocation of NH₃ with 1 m² of litter are an average of 25 mg/h, CO₂ − 8 mg/h, H₂S − 15 mg/h [10]. For non-compliance with growing technologies, emissions of harmful gases can be increased several times and, accordingly, high NH₃ content in poultry air has a negative impact on the keeping and productivity of poultry, the health of the staff, and the ventilation emissions of poultry houses on the environment [11, 12].

Research by many scientists found that one of the important factors that negatively affects the ecology of industrial poultry, is a huge accumulation of microflora in the air, equipment and litter materials of poultry houses [13].

Under favorable microclimate conditions in poultry facilities, the number of microorganisms and their composition is such that between them the state of equilibrium is set, which dynamically changes, but does not affect the health of poultry. However, microbial associations that accumulate excessively in litter materials of the poultry house pose a threat to bird health and its productivity [14]. Extracts of essential oils from plants have long been known for their antimicrobial activity and is an alternative to antibiotics in the cultivation of broiler chickens both in Ukraine and abroad. In vivo studies on broilers demonstrated antimicrobial efficacy of essential oils against Escherichia coli and Clostridium perfringens. So far, the antimicrobial mechanism of the influence of essential oils has not been clarified. However, it is believed that their lipophilic properties and the chemical structure can play their role.

Essential oils of the laurel, cinnamon, thyme and cloves, as well as oregano, basil, coriander and other plants were tested against the bacteria and fungi present in the feed. Oregano essential oils have a high antimicrobial potential, even against gram-negative bacteria, which are usually very resistant to essential oils from other plants [15].

Thus, without proper environmental control and systematic monitoring of the accumulation of pollutants, litter materials pose a potential threat of poultry contamination with pathogenic and opportunistic microorganisms, and in the process of their use from the litter a significant amount of ammonia and other xenobiotics is released, which negatively affect the preservation, productive parameters of a bird and the transformation of feed, and ventilation emissions of poultry houses − to the natural environment.
3. Purpose and tasks of the research

Purpose – carry out chemical and microbiological analysis of litter, investigate the bactericidal properties of essential oils.

To achieve the purpose, the following tasks were set:
1. Sampling of litter broiler production at the initial and final stages of growing poultry breeding;
2. Chemical and microbiological research of litter with broiler growing of poultry;
3. Investigation of bactericidal properties of emulsions of essential oils at concentrations of 0.5 and 1 % on the growth of lactose-positive E. coli and conditionally pathogenic bacteria of the genus Proteus.

4. Materials and methods

The research was carried out at the enterprises of broiler production in the Kiev region. There were sampled 33 samples of litter by the envelope method in the broiler poultry farm – in the initial (10–15 days) and final (37–43 days) period of poultry breeding.

The chemical composition and microbiological studies of litter were carried out in the «Ukrainian laboratory of quality and safety of agricultural products» of the National university of life and environmental sciences of Ukraine and the microbiological laboratory of the Department of Ecology Ltd. "Complex Agromars" according to DSTU ISO 11885:2005 and DSTU 30726-2002.

Appropriate techniques have allowed to investigate the litter for the presence of microorganisms of the genus Salmonella, lactose-positive E. coli and opportunistic bacteria of the genus Proteus, total microbial number and the humidity of the litter.

For studying bactericidal properties of essential oils according to DSTU 50474-93, we prepared agaric selective diagnostic medium (MPB) 5 ml per test tube, added 1 % solution of essential oil emulsion, made in a Petri dish, after which added sown in advance culture of E. coli and Proteus. Strains of the studied microorganisms were provided by the microbiological laboratory of the Department of Ecology Ltd. "Complex Agromars". The emulsion of essential oil was obtained by dissolving it in an organic solvent namely in ethyl alcohol. Essential oils were purchased at a private firm "Leko Style" with an appropriate passport for each. Control was provided by nutrient media with microorganisms, which contained 0,05 ml of distilled water. Thermostated at 37°C 2 days, after which a visual review of the growth of microorganism colonies was conducted.

5. Results of research and their discussion

At the initial stage of growing poultry it was found that in the samples of litter, the moisture content was 22,1 %, and the amount of dry matter was 77,9 % (Table 1).

<table>
<thead>
<tr>
<th>Name of indicators, units of measure, %</th>
<th>Research results</th>
<th>Accuracy studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity</td>
<td>22,1</td>
<td>±0,30</td>
</tr>
<tr>
<td>Dry substance</td>
<td>77,9</td>
<td>±0,30</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>4</td>
<td>±0,30</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>3,1</td>
<td>±0,20</td>
</tr>
<tr>
<td>Total potassium</td>
<td>2,5</td>
<td>±0,10</td>
</tr>
<tr>
<td>Calcium</td>
<td>1,69</td>
<td>±0,02</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0,46</td>
<td>±0,02</td>
</tr>
<tr>
<td>Sodium</td>
<td>0,35</td>
<td>±0,01</td>
</tr>
<tr>
<td>Iron</td>
<td>0,7</td>
<td>±0,0014</td>
</tr>
<tr>
<td>Manganese</td>
<td>0,04</td>
<td>±0,002</td>
</tr>
<tr>
<td>Aluminum</td>
<td>0,04</td>
<td>±0,002</td>
</tr>
<tr>
<td>Zinc</td>
<td>0,04</td>
<td>±0,002</td>
</tr>
<tr>
<td>Strontium</td>
<td>0,005</td>
<td>±0,00001</td>
</tr>
<tr>
<td>Copper</td>
<td>0,004</td>
<td>±0,0001</td>
</tr>
<tr>
<td>Nickel</td>
<td>0,009</td>
<td>±0,0001</td>
</tr>
<tr>
<td>Chromium</td>
<td>0,0002</td>
<td>±0,00003</td>
</tr>
<tr>
<td>Wood sawdust</td>
<td>3,5</td>
<td>±0,30</td>
</tr>
<tr>
<td>Quicklime</td>
<td>7,9</td>
<td>±0,30</td>
</tr>
<tr>
<td>Raw fat</td>
<td>2,25</td>
<td>±0,20</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>7,13</td>
<td>±0,30</td>
</tr>
<tr>
<td>Nitrogen free extract substances</td>
<td>10,62</td>
<td>±0,30</td>
</tr>
<tr>
<td>Amino acids</td>
<td>2,1</td>
<td>±0,20</td>
</tr>
</tbody>
</table>

Since at the final stage of growing poultry, the humidity of the litter increases, accordingly it accumulates more microorganisms, consequently, microbiological studies of litter are more appropriate. In the study of three variants of litter (37, 40 and 43 days of growing poultry) we took into account the moisture of the litter, total microbial number and the presence of microorganisms of the genus Salmonella and lactose-positive E. coli (Table 2).
The above results indicate that the TMN in the first variant of the litter is the smallest of $1.27 \times 10^9$ CFU/g dry matter. The presence of lactose-positive intestinal sticks in 1 g of litter is less than 3, which indicates its proper sanitary condition, while in the other two variants it is absent at all. The evidence of quality production of broiler production is the absence of a pathogenic micro-organism of the genus Salmonella in our studies.

According to [16], bacteria of the genus Salmonella are a causative agent of salmonellosis – the main food-borne disease that the poultry industry faces in the whole world. In total, there are over 2400 serotypes of this microorganism. The most dangerous species for humans is S. typhimurium and S. enteritidis. In broiler production, S. enteritidis is the most common causative agent of food poisoning (33.9 % of cases). In 2015 there were about 95 thousand cases of salmonellosis among people. Foods and water are contaminated through the litter and are the main cause of the spread of the disease in poultry.

S. typhimurium, rarely S. enteritidis, which are very common in nature, often inhabit the gastrointestinal tract of a healthy bird. Salmonella - small mobile gram-negative sticks, spores and capsules do not form. Salmonella is resistant to environmental factors: they are kept in poultry litter for more than 3 months, in the land more than a year, in the corpses buried in the ground – in the summer for 25–40 days, in the winter for 21–31 days in standing water–up to 7 months. They are inactivated at 60°C in 30 minutes, at boiling – after 1 min. under the action of direct sunlight – in 5–9 hours [17].

Actuality of the problem of neutralization of pathogenic and conditionally pathogenic organisms developing in litter in conditions of broiler production is associated with a stable increase in the resistance of key microorganisms to traditional antibacterial agents [18]. One of the promising antibacterial preparations with a wide spectrum of action is essential oils of plants [19].

For the study of bactericidal properties of essential oils, 9 samples of essential oils of such plants were tested: Basil (Ocimum basilicum), Coriander (Coriandrum sativum), Mint pepper (Mentha piperita), Garlic (Allium sativum), Laurel (Laurus nobilis), Cinnamon (Cinnamomum verum), Carnation (Syzygium aromaticum), Lime (Citrus aurantifolia) and Thyme (Thymus) with a concentration of 1 % and 0.5 % of essential oil solution (Table 3).

### Table 2

<table>
<thead>
<tr>
<th>Variants</th>
<th>Humidity, in %</th>
<th>TMN CFU/g dry matter</th>
<th>Coli index CFU/g dry matter</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 days of growing poultry</td>
<td>51.52</td>
<td>$1.27 \times 10^9 \pm 0.2$</td>
<td>$1.6 \times 10^9$</td>
<td>Absent</td>
</tr>
<tr>
<td>40 days of growing poultry</td>
<td>53.45</td>
<td>$1.74 \times 10^9 \pm 0.11$</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>43 days of growing poultry</td>
<td>56.25</td>
<td>$3.39 \times 10^9 \pm 0.35$</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Thus, 7 out of 9 essential oils have bactericidal properties at a minimum concentration of 0.05 ml/5 ml of MPB (1 % concentration of solution). When the volume of the solution increases due to distilled water, the growth of the culture is not suppressed and essential oils lose their bactericidal properties. Therefore, in the future it will be relevant to study the preparation of the drug with a concentration of essential oils to 1 % to determine the optimal-minimum dose for decontamination of the litter from pathogenic microbiota.

### 6. Conclusions

1. Chemical and microbiological analysis of litter has been carried out at the initial and final stages of broiler production, as a result of which it has been shown that in addition to chemical elements and impurities, in the dry matter of the litter there is also an organic matter in the form of raw fat, crude fiber, nitrogen free extract substances and amino acids.

2. It has been shown that there are no pathogenic microorganisms of the genus Salmonella in poultry broiler samples, this testifies to the high quality of products and the satisfactory epidemiological and sanitary state of the investigated poultry enterprise.

3. There are estimated bactericidal properties of essential oils on lactose-positive bacteria E. coli and conditionally pathogenic bacteria P. vulgaris. At a minimum concentration of 0.05 ml / 5 ml of agarized diagnostic-selective medium, 7 of 9 suspensions of essential oils had bactericidal action.

### Table 3

<table>
<thead>
<tr>
<th>Name essential oil</th>
<th>E. coli 1 % solution (0.05 ml EO*/5 ml MPB**)</th>
<th>Proteus vulgaris 1 % solution (0.05 ml EO/5 ml MPB)</th>
<th>Proteus vulgaris 0.5 % solution (0.05 ml EO/10 ml distilled water/5 ml MPB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocimum basilicum</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Coriandrum sativum</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Mentha piperita</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allium sativum</td>
<td>–</td>
<td>+</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Laurus nobilis</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cinnamomum verum</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Syzygium aromaticum</td>
<td>–</td>
<td>+</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Citrus aurantifolia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thymus</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: *EO – essential oil; **MPB – Meat-peptone broth; +++; – – no bacterial growth
References

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