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MICROBICIDAL AND IMMUNOSTIMULATING PROPERTIES OF PLANT-DERIVED SYRUP BASED ON LIME BLOSSOM, ALTHEA ROOT AND THYME EXTRACTS

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The article presents the study's findings aimed at establish microbicidal and immunostimulating properties of the plant-derived syrop based on Lime blossom extract, Althea root extract, Thyme extract. The microbicidal properties of the preparation were examined using the disk-diffusion method, the immunostimulating properties were studied separately and in combination with the live vaccine, which contains the 1/96 strain. 400 thirty-day-old broiler chickens were included in the study and divided into four groups of 100 each: application of the vaccine (Group I) and the plant preparation (Group II), as well as their combined usage (Group III) compared to the control group (Group IV). Results showed an increase of leukocytes by 34.9±0.8, T-lymphocytes by 8.5±0.15, and B-lymphocytes by 6.35±0.8 in the blood of 3rd group chicken broilers, compared to the respective indicators of the control group (24.2±0.5, 1.75±0.20, 1.31±0.15, respectively). So, the preparation has microbicidal and immunostimulating properties in poultry complexes.

Key words: plant immunostimulant, vaccine, infectious bronchitis of chickens, microbicidal properties, antibiotic resistance properties

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МІКРОБІЦИДНІ ТА ІМУНОСТИМУЛЮЮЧІ ВЛАСТИВОСТІ РОСЛИННОГО СИРОПУ НА ОСНОВІ ЕКСТРАКТІВ ЛИПОВОГО ЦВІТУ, КОРЕНЯ АЛТЕЮ І ЧЕБРЕЦЮ

У статті представлені результати досліджень з вивчення мікробіцидних та імуностимулюючих властивостей сиропу рослинного походження на основі екстракту липового цвіту, екстракту кореня алтею, екстракту чебрецю. Мікробіцидні властивості препарату досліджували диско-дифузійним методом, імуностимулюючі властивості вивчали окремо та у поєднанні з живою вакциною, що містить штам 1/96. До дослідження було включено 400 курчат-бройлерів тридцятиденного віку, які були розділені на чотири групи по 100 особин: порівнювали застосування вакцини (1-я група) та рослинного препарату (2-я група), а також їх спільне застосування (3-я група) у контрольній групі (група 4). Результати показали збільшення лейкоцитів на 34,9±0,8, Т-лімфоцитів на 8,5±0,15 та В-лімфоцитів на 6,35±0,8 у крові курчат-бройлерів 3-ї групи порівняно з відповідними показниками контрольної групи (24,2±0,5, 1,75±0,20, 1,31±0,15 відповідно). Так, препарат має мікробіцидні та імуностимулюючі властивості в птахівницьких комплексах.

Ключові слова: рослинний імуностимулятор, вакцина, інфекційний бронхіт курей, мікробіцидні властивості, властивості антибіотикорезистентності.

The widespread dissemination of infectious diseases significantly impacts the global poultry industry, impeding its growth and resulting in substantial economic losses [7, 9].

In scientific literature, extensive data exists regarding the nosological profile of pathogenic microbiota found in birds, particularly focusing on viral and bacterial origins. E. coli, S. enteritidis, P. multocida, S. aureus, coagulase-negative staphylococci, and mycoplasmas are the prevalent causative agents within bacteria. Among viruses, Avian Infectious Bronchitis Virus (AIBV), Chicken Anemia Virus (CAV), Newcastle Virus, and Influenza Viruses stand out as the dominant pathogens IBV still often arises in commercial chicken flocks despite immunization and biosecurity measures because of the ongoing introduction of novel IBV variants and inadequate cross-protection provided by the presently available vaccines [2, 12].

Immunoprophylactic measures have crucial role in combating infectious diseases. However, the pathogenetic properties of diseases and diverse antigenic variation of pathogenic microorganisms can result in diseases even when vaccinations are administered on time. Viewed from this perspective, the continual improvement and maintenance of birds' immune reactivity is a critical concern in combating infectious diseases within the poultry industry [7, 8].

The hypervariability of bacterial and viral strains, along with the rapid development of resistance to antibiotics, necessitates the use of various medications, immunostimulants, and adaptogens during the intensive feeding of birds. Therefore, the development of both microbicidal and immunostimulant drugs has significant scientific and practical importance. Herbal preparations hold a primary position among those utilized for this purpose. They are easily absorbed and do not cause any complications during long-term use [1, 4].

Hence, the accurate and prompt gathering and use of microbicides and immunostimulating preparations derived from medicinal plants are regarded as among the most actual concerns.

The purpose of the study was to assess the microbicidal and immunostimulating efficacy of the plant-derived syrop based on Lime blossom extract, Althea root extract and Thyme extract.

Materials and methods. The antimicrobial properties of Hedalin (Farmabest İlaç Kozmetik Medikal LTD, Turkiye), a plant-derived preparation comprising vitamin complexes (dry extracts of Linden flowers, Thymus vulgaris, chamomile flowers, orange peel, Xanthan gum, and potassium sorbate), were investigated using the disc-diffusion method.

As the test cultures were used microorganism known to cause infectious diseases in poultry farming: Staphylococcus aureus (700699) as representative of Gram-positive bacteria, Escherichia coli (25922) as representative of Gram-negative bacteria, Pseudomonas aeruginosa (1022) and Acinetobacter spp. as nosocomial infection pathogens, Candida albicans (2024) as a yeast strain, Bacillus anthracoides representing Gram-positive spore-forming bacteria, and Klebsiella pneumoniae (505562) as representative of encapsulated bacteria.

Suspensions adjusted to 0.5 MacFarland turbidity were prepared from 24-hour cultures of microorganisms**.** Subsequently, microbial suspensions are poured into Petri dishes containing Meat-peptone agar (MPA) and Sabouraud Dextrose agar (SDA). Sterile discs, saturated with the studied substance for 3-5 minutes, were then placed on the surface of the nutrient medium that had been previously inoculated with microbial culture. 0.89 % physiological solution served as the control. Petri dishes containing MPA were incubated at 37°C, while those with SDA were incubated at 28°C. Results were read after 24–48 hours by measuring the diameter of the inhibition zone formed around the discs.

In order to evaluate the immune stimulating properties of the drug the investigation was conducted on thirty-day-old broiler chickens in the Neftchala, Ujar, and Shabran regions of the Republic of Azerbaijan. Initially, chickens were vaccinated against infectious bronchitis (IB). The primary vaccination for both broilers and breeding birds was administered using the CEVAC® IBird (Ceva Santé Animale (Ceva), Libourne, France) vaccine. CEVAC® IBird is a live attenuated vaccine (LAV) derived from the 1/96 strain belonging to the Bird 793B group and is available in vials containing 500, 1000, or 2500 doses. Immunity develops three weeks after vaccination and remains effective for up to five weeks. The study's main aim was to assess immune status indicators during the use of an immunostimulant alongside IB vaccination of ROZ 308 breed 400 broiler chickens. The birds were selected based on uniform criteria and divided into four distinct groups.

In order to investigate the impact of Hedalin syrup on the immune system during vaccination, 400 birds were divided into four groups, each consisting of 100 birds. Birds were grouped based on their similar characteristics or features. Group I birds received vaccination against IBV with CEVAC® IBird vaccine. Group II birds were administered Hedalin syrup through drinking water. Group III birds received both Hedalin syrup and the CEVAC® IBird vaccine simultaneously. Group IV served as the control - birds were neither vaccinated nor given Hedalin syrup.

The toxicity of the herbal preparation Hedalin was assessed using the toxic-ecological evaluation method. Initially, the drug was dissolved in distilled water and administered orally to white mice at a dose of 20.0 g/kg. These animals were monitored for 15 days, while the control group received only distilled water. Both groups were subjected to identical feeding and storage conditions. The drug's toxicity was evaluated clinically, observing for signs, pathological changes, and mortality among the animals.

Additionally, the safety of the Hedalin preparation was assessed using broiler chickens with the implementation of standard guidelines. The dosage was gradually increased on a daily basis (usually by 2–4 times). The T-lymphocytes were detected in the blood through E-ROK rosette formation with sheep erythrocytes, while B-lymphocytes were detected through spontaneous rosette formation with complement erythrocytes (EA-ROK).

Results of the study and their discussion. The research revealed varying antimicrobial effects of plant-derived syrup on the selected test cultures. Plant-derived syrup demonstrated notable activity against S. aureus, a representative of gram-positive bacteria, with a 14 mm inhibition zone, and C. albicans, with a 12 mm inhibition zone. Its most significant antimicrobial effect was observed against Escherichia coli – gram-negative bacteria, generating an 18 mm inhibition zone. The weakest effect was against representatives of encapsulated bacteria – Klebsiella pneumoniae (3 mm) and B.anthracoides (6 mm).

Considering the emergence and spread of new AIB serotypes, especially the 1/96 strain in Azerbaijan, it's crucial to account for these serotypes in vaccine formulations used for bird vaccination.

No toxic symptoms associated with plant-derived syrup were observed during the study. Additionally, no pathological changes were noted in the internal organs during the autopsy of both mice and chickens subjected to the treatment.

In order to study the effect of plant-derived syrup on the immune system during vaccination, 400 birds were grouped into 4 categories, each comprising 100 birds. Group I received the vaccine through

drinking water, and group II was given plant-derived syrup via drinking water to observe its effect on the immune system. Group III, birds received plant-derived syrup alongside vaccination. For this group, the drug was administered for 3 consecutive days before vaccination, followed by vaccination, and then the immunostimulant was given again on the 5th, 6th, and 7th days post-vaccination.

The control group (IV) birds were neither vaccinated nor administered plant-derived syrup. Three weeks post-vaccination across all groups, blood was taken from the subclavian vein of the birds for immunological analyses. A significant increase in leukocyte and lymphocyte counts was observed upon evaluating the immunological status of chickens in the experimental groups. The leukocyte count for Group I was $32.9\pm0.8x10^3$/µl, $p<0.001$; for Group II, it was $23.2\pm0.5x10^3$/µl, $p<0.001$; for the control, it was $24.2\pm0.5x10^3$/µl, $p<0.05$ (vs. control group); for Group III, it was $34.9\pm0.8x10^3$/µl, $p<0.01$; and for Group IV, it was $24.2\pm0.5x10^3$/µl. Additionally, an increase in lymphocyte count was observed across all experimental groups (Table 1).

Table 1

Results of experimental research

Experimental groups		Leukocytes (10^3/µl)	Lymphocytes	
			T (10^3/µl)	B (10^3/µl)
Group I	Vaccine	32.9±0.8***	7.9±0.5**	5.9±0.20***
Group II	Plant-derived syrup	23.2±0.5***	5.1±0.42***	2.9±0.18***
Group III	Plant-derived syrup +vaccine	34.9±0.8**	8.5±0.15***	6.35±0.8**
Group IV	Control	24.2±0.5*	1.75±0.20	1.31±0.15**

Note: * p< 0.05; ** p< 0.01; *** p< 0.001

The T lymphocyte count was $7.9\pm0.5x10^3$/µl in Group I, $5.1\pm0.42x10^3$/µl in Group II, $8.5\pm0.15x10^3$/µl in Group III, and $1.75\pm0.20x10^3$/µl in the control group. Meanwhile, the count of B lymphocytes was $5.9\pm0.20x10^3$/µl in Group I, $2.9\pm0.18x10^3$/µl in Group II, $6.35\pm0.8x10^3$/µl in Group III, and $1.31\pm0.15x10^3$/µl in the control group.

The administration of plant-deriver syrop based on Lime blossom extract, Althea root extract, and Thyme extract in combination with the live attenuated vaccine derived from the 1/96 strain resulted in a noticeable rise in lymphocyte count. This demonstrates the syrup's substantial microbicidal effect, leading to the absence of opportunistic infections and negating the need for antibiotics throughout the study. So, specific preventive use of plant-derived syrup based on Lime blossom extract, Althea root extract, and Thyme extract significantly elevated the birds' immune response against infectious bronchitis, evidenced by increased T and B lymphocyte levels in the blood during the experiments. Apart from its immune stimulating impact, this plant-derived syrup we used (both isolated and simultaneously with vaccine) also exhibits microbicidal properties.

Nowadays, the poultry industry contributes significantly to bridging the nutritional gap in many countries because its meat and egg products are rich in protein and valuable nutrients at a cost less than other animal meat sources. The natural antibiotic alternatives, including probiotics, prebiotics, symbiotics, organic acids, essential oils, enzymes, immunostimulants, and phytogenic (phytobiotic), including herbs, botanicals, essential oils, and oleoresins, are the most common feed additives that acquire popularity in poultry industry following the ban of antibiotic growth promoters (AGPs). They are widely used throughout the world due to their positive impact on poultry farming. They are easily mixed with other feed ingredients, do not have tissue residues, improve feed intake, feed gain, feed conversion ratio, improve poultry immunity, improve digestion, increase nutrient availability and absorption, have an antimicrobial effect, do not affect carcass characteristics, reduce the use of antibiotics, act as antioxidants, anti-inflammatory agents, fight stress factors and provide healthy organic foods for human consumption [1, 4, 6].

Several studies were emphasized that naturally derived enzymes, antioxidants, and botanicals can be used in organic poultry farming to combat infections, improve growth and enhance the quality of the products [4, 10, 13].

Additionally, vaccines are allowed against many different diseases as Marek's disease virus, Newcastle disease virus, infectious bronchitis virus, Mycoplasma, and coccidia [5, 9].

Ashour et al., (2021) noted that in cases with improving immunity a broad category of factors commonly researched in poultry is plant-derived additives (phytogenic feed additive, PFA) intended to improve gut health and function. PFAs are plant-derived compounds such as essential oils, spices, safe natural compounds, and herbs intended to provide a health benefit when added to feed [3]. Among them, typical examples are rosemary derivatives, oregano, thyme, sage, cinnamon, citrus, pepper, and anise [10]. The supplementation of a PFA containing star anise as one of its lead active components improved nutrient digestibility. Mentha extracts in particular, have been reported to improve digestibility, via the control of microbial growth in the intestine. Therefore, the improvement of the aforementioned growth performance

parameters in the present study could partly be explained in the light of improvements in the digestibility of dry matter.

Ferdous et al., (2019) showed that the efficacy of phytogenics can depend on numerous factors, including composition, inclusion level in the feed, bird genetics, and feed composition [6].

Paraskeuas V., et al (2016) in their study demonstrated increased ileal IgA levels and a trend towards a decrease in spleen IL-18 of d 42 broilers fed a PFA mixture (photogenic characterized by menthol, anethole and eugenol). The authors assessed the impact of this phytogenic on broiler growth performance biochemical parameters, including total antioxidant capacity and gene expression. The same study using wheat instead of corn observed no differences in ileal IgA or spleen IL-18. According to the authors results, their study provides evidence for the beneficial role of phytogenics as a natural growth promoter with the potential to favour broiler chickens' health. Further research is required to assess PFA effects on immune responses and gut microbiota indices under a variety of diet formulations combined or not with pathogenic challenge [11]. In our study, we evaluated the phytogenic with different compositions, so we assessed the blood count parameters. Besides this, in the study mentioned above, one-day-old male Cobb broiler chickens were involved, and the duration of the experiment was 42 days. In our experiment, the thirty-day-old broiler chickens were the subject, and the duration of monitoring was shorter – 15 days.

Cho J.H., et al (2014) used star anise essential oils to evaluate its effect on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with Clostridium perfringens in broilers and got positive results [4]. So, it is considered that the problem we are working on needs additional investigations.

Conclusion

Significant increase in leukocyte counts in groups used plant-derived syrup based on Lime blossom extract, Althea root extract, and Thyme extract was observed. The leukocyte count for a group with isolated use the syrup was $23.2\pm0.5x10^3/\mu l$ ($p<0.001$ in comparison to control). Additionally, an increase in lymphocyte count was observed across all experimental groups. So, specific preventive use of plant-derived syrup based on Lime blossom extract, Althea root extract, and Thyme extract notably elevated the birds' immune response against infectious bronchitis, evidenced by increased T and B lymphocyte levels in the blood during the experiments. Notably, apart from its immune stimulating impact, this plant-derived syrup also exhibits microbicidal properties.

References

1. Abd El-Hack ME, El-Saadony MT, Salem HM, El-Tahan AM, Soliman MM, Youssef GBA, et al. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. Poult Sci. 2022 Apr;101(4):101696. doi: 10.1016/j.psj.2022.101696.

2. Abozeid HH., Paldurai A., Khattar SK., Afifi MA, El-Kady MF, El-Deeb A. H, et al. Complete genome sequences of two avian infectious bronchitis viruses isolated in Egypt: Evidence for genetic drift and genetic recombination in the circulating viruses. Infect. Genet. Evol. 2017; 53: 7–14. doi: 10.1016/j.meegid.2017.05.006

3. Ashour EA, Farsi RM, Alaidaroos BA, Abdel-Moneim AME, El-Saadony MT, Osman AO, et al. Impacts of dietary supplementation of pyocyanin powder on growth performance, carcase traits, blood chemistry, meat quality and gut microbial activity of broilers. Ital. J. Anim. Sci. 2021; 20:1357–1372.

4. Cho JH, Kim HJ, Kim IH. Effects of phytogenic feed additive on growth performance, digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with Clostridium perfringens in broilers. Livest Sci. 2014; 160: 82–88.

5. El-Naggar MS, Ibrahim HM, Salem HM, Marouf S. A novel locally prepared inactivated bivalent mycoplasma vaccine for chicken flocks in Egypt. Adv. Anim. Vet. Sci. 2022; 10: 55–61.

6. Ferdous MF, Arefin MS, Rahman MM, Ripon MMR, Rashid MH, Sultana MR, et al. Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. J Adv Vet Anim Res. 2019 Aug 20;6(3):409–415. doi: 10.5455/javar.2019.f361.

7. Fischer S., Klosterhalfen D., Wilms-Schulze Kump F., Casteel M. Research Note: First evidence of infectious bronchitis virus Middle-East GI-23 lineage (Var2-like) in Germany. Poult. Sci. 2020; 99: 797–800. doi: 10.1016/j.psj.2019.10.031

8. Heylen D, Bisaglia B, Fracasso G, Prinsen E, Müller W, Matthysen E. Ineffective humoral anti-tick IgY-response in birds: reaction against pathogen constituents? Open Res Eur. 2021 Sep 23;1:8. doi: 10.12688/openreseurope.13204.2.

9. Marouf S, Moussa IM, Salem H, Sedeik M, Elbestawy A, Hemeg HA, et al. A picture of Mycoplasma gallisepticum and Mycoplasma synoviae in poultry in Egypt: Phenotypic and genotypic characterization. J. King Saud Univ. Sci. 2020; 32: 2263–2268.

10. Mountzouris KC. Phytogenic and probiotic feed additives for broilers: evidence for growth performance links with gut performance indices. Pages 107–116 in Proceedings of the 2016 World Nutrition Forum. Pages 107-116 in Proceedings of the 2016 World Nutrition Forum; Austria; Erber Ag; 2016.

11. Paraskeuas V, Fegeros K, Hunger C, Theodorou G, Mountzouris KC. Dietary inclusion level effects of a phytogenic characterized by menthol and anethole on broiler growth performance, biochemical parameters including total antioxidant capacity and gene expression. An. Prod. Sci. 2016; 57: 33–41.

12. Rafique S, Jabeen Z, Pervaiz T, Rashid F, Luo S, Xie L, Xie Z. Avian infectious bronchitis virus (AIBV) review by continent. Front Cell Infect Microbiol. 2024 Feb 5;14:1325346. doi: 10.3389/fcimb.2024.1325346.

13. Setta A, Salem HM, Elhady M, El-Hussieny A, Abdel Satar Arafa A. Molecular and genetic characterization of infectious bronchitis viruses isolated from commercial chicken flocks in Egypt between 2014 and 2016. J. World Poult. Res. 2018; 8:1–8.

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