Effect of *Satureja montana* essential oil on the bactericidal activity of broiler chickens blood serum

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**Abstract.** Essential oils as a feed additive in animal and poultry nutrition should preserve the bactericidal properties of blood and suppress the formation of bacterial biofilms. The study of bacterial biofilms and bacterial activity of blood serum in broiler chickens against the background of the addition of essential oil of *Satureja montana* to a diet was discussed in the article. Blood serum of broilers that received essential oil with feed suppressed the growth of the bacterial biofilms at *S. aureus* test culture by 60-72%. It serum had a weaker effect on the bacterial biofilms at *E. coli* test culture; it reduced the density by 23.5%. This effect began only after the 10th day of inclusions of essential oil. The blood serum of chickens that received essential oil in the form of aqueous solutions had a weak antimicrobial effect against growing the bacterial biofilms at *S. aureus* test culture; the density was 10% lower than that of the control. The growth of the bacterial biofilms of *E. coli* test culture was not inhibited.

**1 Introduction**

Bacterial biofilms is a highly organized supracellular system consisting of a continuously changing heterogeneous community of bacteria and an associated extracellular exopolymer matrix. Each of the components contributes to the formation of antibiotic resistance of bacterial biofilms in this structural and functional organization. Microorganisms form groups of persistent cells that are immune to external influences [1].

The formation of biofilms is one of the factors of pathogenicity of microorganisms, so most infectious processes begin with their formation [2].

Bacterial biofilms are a big problem in biology, which can appear on intact areas of the human body, in wounds; they can cover the surfaces of catheters, artificial implants, prostheses, etc. The degree of adhesion with the subsequent formation of biofilms is the most pronounced to such materials like latex, silicone, polyvinyl chloride [3]. The pathogenesis of infections is determined by microbial biofilms and is called biofilm infections [4].

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However, the growth of microorganisms in the form of biofilms can ensure their positive use in processes related to biotransformation of substances in pharmacology, food production, wine-making, etc.; for biological treatment of water, air, and soil; in the biological control of phytopathogens that cause plant diseases; in medicine and veterinary medicine when using preparations of live bacteria with probiotic activity [5].

The blood can delay the growth or cause the death of many types of microorganisms. These properties of blood and its serum can dissolve bacterial cells [6]. Blood serum is able to destroy the bacterial biofilms; blood serum is one of the factors of humoral non-specific resistance also. Serum bactericidal activity (SBA) is an integral indicator of the level of non-specific resistance of the body [7].

Because of in vitro studies, antibiotics do not show the same results in animals as the use of essential oil. This is probably due to the blocking effect of blood serum components on antibiotics, as indicated in the article by Nikolaevsky et al. [8]. No similar effect was observed when using essential oils.

Therefore, adding essential oils to animal feed or water should preserve the bactericidal properties of blood and suppress the formation of bacterial biofilms. The study of bacterial biofilms and bacterial activity of blood serum in broiler chickens against the background of the addition of essential oil of Satureja montana to a diet of broilers of Cobb-500 cross was the aim of our research.

2 Materials and methods

Cobb-500 broilers cross was the object of the research. Hens were housed in cages. Feeding (main diet) - ready-made full-ration feed mixes to meet nutrient requirements. A total number of broiler chickens were divided into three groups: I (control); II (first experimental group); III (second experimental group) The control group received main diet or MD (no supplement) throughout the experimental period. Both experimental groups were, respectively, fed diets containing MD + mountain savory essential oil at a rate of 0.2 ml per 30 heads; MD + mountain savory essential oil diluted 50 mg/l. We evaluated bacterial biofilms (BBF) and serum bactericidal activity (SBA).

Optical density (E) of the serum was determined on a photoelectric colorimeter using a green light filter. Distilled water (3 mm cuvette) served as a control variant (in a parallel beam of light). Meat-peptone broth in the volume of 4.5 ml was used for setting the reaction. Test cultures - Escherichia coli and Staphylococcus aureus.

Preparation of microbial culture: daily agar culture by washing was adjusted to 2 billion m3 cells per 1 ml of a sterile physiological solution on a photoelectric colorimeter. Then 0.01 ml of microbial suspension was added to 4.5 ml of meat-peptone broth and left in the thermostat for 24 hours. Both in the experiment and in the control variant, 0.1 ml of daily broth culture was added to the test tubes.

In test tubes with 4.5 ml of distilled water, 1 ml of the tested serum of sterile meat-peptone broth was added. Then 0.1 ml of daily culture of Escherichia coli or Staphylococcus aureus was also added to all test tubes. Test tube with meat-peptone broth without serum served as a control in this experiment. The contents of the test tubes were thoroughly mixed and 2 ml was taken from each sample with a sterile pipette to measure the optical density. The mixture that had left in the test tubes (meat peptone broth + serum + microbe culture) was placed in the thermostat at 37.0 °C for 3 hours. We obtained two indicators: the first one characterized the optical density of meat-peptone broth with culture and serum immediately after mixing; the second showed the optical density of the same mixture after 3 hours of incubation in the thermostat.

SBA expressed in units of inhibition of growth and development of microbes according to the formula 1:
\[ SBA = \frac{Eod_3 - Eod_0}{Ec_3 - Ec_0}, \]  

where \( SBA \) - conventional unit;  
\( Eodo \) - optical density of the test sample before incubation;  
\( Eod3 \) - optical density of the test sample after 3 hours of incubation;  
\( Ec0 \) - optical density of the control sample before incubation;  
\( Ec3 \) - optical density of the control sample after 3 hours of incubation.

Effect of poultry serum on the density of BBF of opportunistic bacteria \( S. aureus \) ATCC 25923 and \( E. coli \) ATCC 25922 in test culture was used. Biofilms of daily bacterial cultures were obtained in a 96-well polystyrene tablet. Cultures were incubated in wells with a nutrient medium and serum at 37° C for 24 hours to produce a mature BBF. The suspension part of the cultures was removed. The density of biofilms was estimated using the O'toole & Kolter' method on a Multiskan spectrophotometer at a wavelength of 620 nm by the intensity of staining, bound by the BBF and extracted by ethanol gentian violet. The serum was studied in a dilution of 1:10. Options without adding the serum served as control. The same volume of sterile isotonic sodium chloride solution was added to the control variants to maintain an identical volume.

3 Research results

Serum bactericidal activity of broilers when diluting serum 1:10 in the \( S. aureus \) test culture is shown in fig. 1. The growth of bacteria to negative values in the first three hours of exposure was strongly suppressed to -37.3...-12.9%. SBA remained at the same level in the following hours of exposure: from 20.6 to 29.8%. The blood serum of the chickens from the control group reduced its bactericidal activity almost twice in comparison with the serum of the experimental group to 58.2% at the seventh hour of exposure.

![Fig. 1. Serum bactericidal activity of broilers when diluting the serum 1:10 in S. aureus test culture.](image)

SBA of broilers when diluting serum 1:5 in \( S. aureus \) test culture is presented in fig. 2. The bactericidal activity of blood serum remained at a low level from the fifth hour of exposure.
Gradual suppression of microflora during exposure from 84.1 to 3.3% against the background of the test culture of *E. coli*, with its 1:10 dilution, was noted. In the control group, this indicator was less pronounced: from 67.7 to 10.4%, respectively (Fig. 3).

The effect of *E. coli* test culture at 1:5 dilution was not observed (Fig. 4). The blood serum of both groups of chickens remained bactericidal throughout the entire exposure period, suppressed bacteria for the first three hours to negative values during this period: -40.8...-53.2%.

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**Fig. 2.** Serum bactericidal activity of broilers when diluting the serum 1:5 in S. aureus test.

**Fig. 3.** Serum bactericidal activity of broilers when diluting the serum 1:10 in *E. coli* test culture.
Fig. 4. Serum bactericidal activity of broilers when diluting the serum 1:5 in E. coli test culture.

The results of the action of chicken serums in a 1:10 dilution on the growth of Staphylococcus aureus and Escherichia coli bacterial biofilms are presented in table 1.

Table 1. Effect of broiler chicken cross Cobb-500 serums on the growth of S. aureus and E. coli bacterial biofilms, in % to the control group.

| Group                  | The bacterial biofilm density |
|------------------------|------------------------------|
|                        | S. aureus test culture | E. coli test culture |
| First (control)        | 90.1                        | 119.8                  |
| Second (experimental)  | 89.5                        | 152.6                  |
| Third (experimental)   | 39.6                        | 109.9                  |

a Value of the optical density of BBF in the control is assumed to be 100%. Values less than 100% indicate suppression of BBF growth.

In broilers of the control group, the serum had an inhibitory effect on the growth of S. aureus test culture. More pronounced suppressing effect on BBF growing had serums of chickens that received essential oil with feed compared to broilers that received essential oil with water.

The density of E. coli BBF during incubation with chicken serum was higher than the control values. Adding essential oil to the feed increased the bactericidal activity of chicken serum against BBF growing: there was some positive dynamics of this activity for 10 days. E. coli BBF was suppressed by 23.5% in comparison with the control variant in this case. Serum activity indicators for E. coli BBF were significantly lower when using essential oil with water. Only after 30 days of applying essential oil as a solution, the density of BBF during incubation with chicken serum was not higher than the control values.

The antimicrobial effect of wormwood essential oils on biofilm formation by C. epidermal, staphylococci, E. coli, and C. albicans fungi has been studied. Essential oils of wormwood with antibacterial action were isolated are next species: Artemisia obtusiloba ledeb., Artemisia obtusiloba Ledeb., Artemisia santolinifolia Turcz., Artemisia glauca Pall. and Artemisia sieversiana Willd. However, no essential oils have been identified that have a
fungicidal effect [9]. The role of microbial biofilms in the development of human infections has been proved [10]. Thus, it is necessary to evaluate not only the antimicrobial effect of drugs, but also their effect on the biofilm formation of microorganisms S. Epidermidis and E. coli to the formation of biofilms. The greatest effect is to suppress the formation of biofilms of C. albicans [11-13].

Monoterpenoids, sesquiterpenoids, flavonoids and coumarins, and aliphatic and lipid compounds are part of essential oils [14]. These compounds have an effect on biofilms. The essential oil of A. annua was found to be antiinflammatory, antipyretic [15], anticancer [16], antifungal [17], antiparasitic [18], antitumor [19], and cytotoxic [20] effects. Chemical variability of the composition of essential oil depends on the geographical origin and stage of plant development [14, 21]. First report on antimicrobial and antioxidant activity of A. Appia essential oil growing in Bosnia, reported by Cavar Sanja et al. [22]. Antimicrobial activity of A. appia has been reported against various microorganisms- E. coli, S. aigeas, Candida albicans [16, 23].

4 Conclusions

Blood serum of broilers that received essential oil with feed suppressed the growth of S. aureus BBF by 60-72%. These serums had a weaker effect on E. coli BBF (it reduced the density by 23.5%); the effect was noticeable after the 10th day of applying the essential oil. Serums of chickens that received essential oil in the form of aqueous solutions had a weak antimicrobial effect against growing S. aureus BBF: the density is 10% lower than the control values. The growth of E. coli BBF was not inhibited.

References

1. Hall-Stoodley L and Stoodley P 2009 Evolving concepts in biofilm infections Cell. Microbiol. 11(7) 1034–1043
2. Costeron J W 1999 Bacterial Biofilms: A Common Cause of Persistent Infections Science 284 1317-1322 DOI: 10.1126/science.284.5418.1318
3. Darouiche R O 2001 Device-associated infections: a macroproblem that starts with microadherence Clin. Infect. Dis. 33(9) 1567–1572
4. Romling U and Balsalobre C 2012 Biofilm infections, their resilience to therapy and innovative treatment strategies J. Intern. Med. 272(6) 54–561
5. Donlan R M and Costerton J W 2002 Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms Clin. Microbiol. Rev. 15(2) 167–193
6. Gumus D, Kalayci-Yuksek F, Uz G, Bilgin M and Ang-Kucuker M 2018 The Possible Effects of Different Hormones on Growth Rate and Ability of Biofilm Formation in Different Types of Microorganisms Acta Microbiologica Bulgarica 34(1) 47–51
7. Slivinska L G, Fedorovych N M 2012 Application chelates microelements in young animal sheep Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies (Veterinary Sciences) 15(3) 57(1) 283–286
8. Nikolayevsky V V, Eremenko A E and Ivanov I K 1987 Biological activity of essential oils (Moscow: Medicine Publishing House) p 144
9. Kartashova O L, Tkachev AV, Utkina T M and Potekhina L P 2012 Influence of essential oils of wormwood of microorganism growth and biofilm formation Bulletin of the Orenburg scientific center of the Ural branch of the Russian Academy of Sciences (electronic journal) 3 1–10
10. Casterton J W, Stewart P S and Greenberg E P 1999 Bacterial biofilms: a common cause of persistent infections Science 284 1318–1322

11. Bachmann S P, Walle K V, Ramage G et al 2002 In vitro activity of caspofungin against Candida albicans biofilms Antimicrob. Agents Chemother 46(11) 3591–3596 DOI: 10.1128/AAC.46.11.3591-3596.2002

12. Silva S, Rodrigues C F, Araújo D, Rodrigues M E and Henriques M 2017 Candida Species Biofilms’ Antifungal Resistance Review J Fungi 3(1) 8 https://doi.org/10.3390/jof3010008

13. Vediyappan G, Rossignol T and D'Enfert C 2010 Interaction of Candida albicans Biofilms with Antifungals: Transcriptional Response and Binding of Antifungals to Beta-Glucans Antimicrob. Agents Chemother 54(5) 2096–2111

14. Bhakuni R S, Jain D C and Sharma R P 2002 Phytochemistry of Artemisia annua and the development of artemisinin-derived antimalarial agents Artemisia ed C W Wright (London: Taylor & Francis) 211–248

15. Huang L, Liu J F, Liu L X, Li D F, Zhang Y, Nui H Z, Song H Y and Zhang C Y, 1993 Antipyretic and anti-inflammatory effects of Artemisia annua L. Zhongguo Zhong Yao Za Zhi 18(1) 44–48

16. Zheng G Q 1994 Cytotoxic terpenoids and flavonoids from Artemisia annua Plant Med. 60(1) 54–57

17. Liu C H, Zou W X, Lu H and Tan R 2001 Antifungal activity of Artemisia annua endophyte cultures against phytopathogenic fungi J. Biotechnol. 88(3) 277–282

18. Kim J T, Park J Y, Seo H S, Oh H G, Noh J W, Kim J H, Kim D Y and Youn H J 2002 In vitro antiprotozoal effects of artemisinin on Neospora caninum Vet. Parasitol 103(1-2) 53–63

19. Foglio A, Possenti A, Nogueira D C F and de Carvalho J E 2001 Antiulcerogenic activity of crude ethanol extract and some fractions obtained from aerial parts of Artemisia annua L. Phytother. Res. 15(8) 670–675

20. Nibret E. and Wink M. 2010 Volatile components of four Ethiopian Artemisia species extracts and their in vitro antitrypanosomal and cytotoxic activities Phytomedicine 17(5) pp. 347–369

21. Lenardis A E, Morvillo C M, Gil A and de la Fuente E B 2011 Arthropod communities related to different mixtures of oil (Glycine max L. Merr.) and essential oil (Artemisia annua L.) crops Ind. Crop. Prod. 34(2) 1340–1347

22. Sanja C, Maksimovic M, Vidica D and Pari A 2012 Chemical composition and antioxidant and antimicrobial activity of essential oil of Artemisia annua L. from Bosnia Industrial Crops and Products 37 479–485

23. Duarte M C T, Leme E E, Delarmelina C, Soares A A, Figueira G M and Sartoratto A 2007 Activity of essential oils from Brazilian medicinal plants on Escherichia coli J. Ethnopharmacol 111(2) 197–201