Biological evaluation of aqueous extract of oak bark on in vitro models

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Abstract. The article presents the results of studies on the biological activity and effectiveness of drug administration of oak bark extract in various concentrations and mixed with native cicatrificial fluid on the luminescence dynamics of a lux biosensor - a genetic engineering strain of Escherichia coli K12 TG1, a constitutively expressing luxCDABE genes of a natural marine microorganism Photobacterium leiognathi 54D10. It has been established that the administration of oak bark extract, in ratios: 1:12, 1:10, 1:8, (dry matter of bark to distilled water), does not have a toxic effect on the culture of E. coli K12 TG1. The dynamics of feed digestibility after three and six hours of exposure when making oak bark extract in different dosages is accompanied by an increase in the total digestibility coefficient, after 3 hours by a value from 3.92% (P≤0.001) to 21.18% (P≤0.001), after 6 hours the digestibility increases 3.18% (P≤0.001) to 24.25% (P≤0.001) relative to the control. The greatest digestibility is achieved in a dose of 150 µl. Also, a relationship was found between the dose of oak bark extract and the percentage of digestibility: the higher the dosage, the lower the digestibility, and vice versa - the higher the dosage, the lower the coefficient of digestibility of the dry matter of the feed substrate.

1. Introduction

Plant materials are widely used in traditional medicine systems [1], also in feeding farm animals, to stimulate antimicrobial, anti-inflammatory, antioxidant and antiparasitic activity [2]. Many plants have useful multifunctional properties and the bioactive components derived from them can favorably affect the animal's organism [3], serve as a growth stimulator and protect the animal's organism [4,5]. It is also reported on the effect of plant extracts on the increase in live weight of the bird [6].

Plant extracts are generally considered safe and effective against pathogenic bacteria, with minimal inhibitory concentrations of 100–1000 µg / ml in in vitro bacterial susceptibility tests possess antibacterial activity [7].

A common feature of herbal extracts is that they are a complex mixture of biologically active components. The composition of the extracts can vary due to biological factors: the place of cultivation, collection conditions, extraction and storage conditions [8] Only under certain circumstances, plant extracts can improve animal digestion and control microflora [9]. Due to the complex composition of plants and their extracts, it is difficult to conduct a comprehensive toxicity study and safety assessment. The task is to determine the optimal amount of substances contained in the plant extract, beneficially affecting the bioavailability of diets that do not violate the physiology of animals, but due to an increase
in the productivity of beef cattle [10].

Currently, the biotest method is used to solve the problems of analyzing the toxicity of substances of complex composition. This method consists in determining the toxicity of substances directly under the action on a living organism [11].

When using herbal feed additives, you need to consider their interactions with other nutrients in the diet [12].

Thus, herbal preparations are a group of natural supplements that serve as alternatives to stimulate animal growth, but studies of their mechanism of action, interactions with other components of the diet, toxicity and safety assessment require further study before they can be used in feeding farm animals [13]. In this regard, the purpose of this study was to study the biological effect of the preparation of oak bark extract in the bacterial bioluminescence inhibition test (Echerichia coli) with cicatricial fluid, as well as the analysis of the dynamics of digestibility of the feed substrate.

2. Materials and methods

Object of study. Gobies at the age of 13 months; red steppe breed; scar fluid.

Animal care and experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 on 08/12/1977, the USSR Ministry of Health) and "The National Academy Press, Washington, DC 1996). In doing research, efforts were made to minimize animal suffering and reduce the number of samples used.

The scheme of the experiment. The studies were conducted on the basis of the Center for Nanotechnology in Agriculture of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences.

As the herbal preparation was used aqueous extract of oak bark (EOB) in the ratio: 1:12; 1:10; 1:8 JSC "Krasnogorskleksredstva", Krasnogorsk, Russia.

Obtaining aqueous extract of oak bark. Into 25 ml plastic centrifuge tubes weighed 1 gram. Distilled water in a volume of 12, 10, 8 ml was ground into powdered oak bark. Then they were incubated in a water bath (LOIP LB-140, Sibagropribor, RF) for 30 minutes at a temperature of 80 °C, centrifuged at 2000 rpm (OPN-8 centrifuge, manufacturer ZAO Tehnomok, RF), filtered and adjusted to a certain volume distilled water.

The composition of the extract was determined by high-performance liquid chromatography on a gas chromatograph with a GQCMS 2010 Plus mass-selective detector (Shimadzu, Japan) on an HP-5MS column [14].

Biological evaluation of oak bark extract (EOB) is given on the model of the genetically engineered luminescent strain of Echerichia coli K12 TG1, constitutively expressing lux CDABE genes of the natural marine microorganism Photobacterium leiongnathi 54D10, produced by the NWO Immunotech (Moscow, Moscow), was in a lyophilized condition, it was in a real weight, it was a condition, it was in a state, it was in a state, it's " . Immediately before conducting the studies, this preparation was restored by the addition of chilled distilled water and standardized to an optical density of 0.3 at a wavelength of 600 nm. The suspension of bacteria was kept at a temperature of 2-4 °C for 30 minutes, after which the temperature of the bacterial suspension was adjusted to 15-25 °C.

The test was carried out using the recommendations of D.G. Deryabina [15]. Test cells and a suspension of luminescent bacteria were introduced into the cells of 96-well plates in a 1: 1 ratio, after which the tablet was placed in the measuring unit of the Infinite PRO F200 microplate analyzer (TECAN, Austria), which recorded the luminescence intensity of the mixtures obtained for 180 min with an interval of 3 min The results of the effect of the preparations of the UDC on the intensity of bacterial bioluminescence were evaluated using formula (1).

\[ I = \frac{I_{0} min \times I_{n} min}{I_{n} min \times I_{0} min} \tag{1} \]

Ik and Io are the luminescence intensity of the control and experimental samples at the 0th and nth minutes of measurement.
The dynamics of digestibility was carried out according to GOST 24230-80 Plant feed. Method for the determination of digestibility in vitro. Samples of crushed air-dried wheat bran weighing 500 mg and oak bark extract in the ratio (1:10) at a dose of 200 μl, 150 μl, 100 μl, control. Placed in test tubes, pre-dried to constant weight, poured 50 cm of scar fluid with phosphate buffer solution in a ratio of 1:4, closed with stoppers and kept in a thermostat (TS-1/80 SPU, OAO Smolensk SKTB SPU) at \( t = 39 ^\circ C \). After a certain time of 3, 6 hours, the supernatant was removed with a water jet pump (Rocker 300, Taiwan) and washed with distilled water, centrifuged in an OPN-8 centrifuge for 5 minutes at a rotational speed of 2500 minutes, and the supernatant was again removed. The tubes with undigested residues are placed in a drying cabinet (GC SUP-4 WAMED, Poland), dried at 100-105 ° C to constant weight, and after cooling in a desiccator, weighed on a scale (VM 153, OKB Vesta LLC, RF). Hygroscopic moisture of wheat bran was determined by the difference in mass before and after drying according to GOST 31640-2012. The coefficient of digestibility of dry matter feed (x) in percent was calculated by the formula (2).

\[
x = \left[ \frac{m \times (100-m_2) - m_1}{m \times (100-m_2)} \right] \times 100
\]

\( m \) is the weight of the feed, mg; \( m_1 \) is the mass of the dried undigested food residue, mg; \( m_2 \) - mass fraction of hygroscopic moisture in the feed, %.

Statistical processing: Data are expressed as mean values ± standard error of the mean. Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). Significance of the group differences was estimated using Student’s t-test with \( p \leq 0.05 \) considered as significant.

3. Results
In studies, induction of Elcherichia coli K12 TG1 cells was observed upon contact with cicatricial fluid, which developed in the first 20 min of contact and was observed throughout the experiment (Fig. 1). It is possible that the development of luminescence induction is associated with the influence of some one or several components in the cicatricial fluid, which act as a nutrient substrate for bacterial cells, which has accelerated metabolic processes.

![Figure 1](image-url)

**Figure 1.** The luminescence dynamics of E. coli K12 TG1 with cloned luxCDABE genes of P. leiongnathi 54D10 upon contact with cicatricial fluid (1) and successive 6-fold dilutions of cicatricial fluid (2-7); to - control.
Oak bark extract at various concentrations is marked by the absence of a toxic effect on bacteria cells (Fig. 2a). Testing the combination of the extract with cicatrical fluid using the E. coli K12 TG1 strain showed a slight induction of the luminescence of the bacterial cells in comparison with the control (Fig. 2b).

The bioluminescent process is regulated by the metabolism of bacteria and the stage of their growth. The emitted light decreases when the bacteria are in sub-optimal conditions of temperature, nutrition, and so on.

Thus, similar effects on bacterial bioluminescence of oak bark extract and combination of cicatrical fluid were recorded, and they proved to be non-toxic.

To study the dynamics of digestibility, we used aqueous oak bark extract in the ratio of 1:10. When making oak bark extract in different dosages, the digestibility of dry matter of wheat bran after 3, 6 hours of exposure increases with respect to the control (Fig. 3).
Figure 3. The dependence of the digestibility of dry matter on the dosage of oak bark extract at an exposure of 3 and 6 hours.

After 3 hours of exposure, the greatest digestibility is achieved with the introduction of oak bark extract in a dosage of 150 µl, which is 21.18% (P≤0.001) more control. With the introduction of a minimum dose of 100 µl of oak bark extract, the digestibility increases by 3.92% (P≤0.001).

With a 6-hour exposure, the tendency is maintained, as with the introduction of the maximum dose of ECD 200 µl, the digestibility increases significantly by 12.47% (P≤0.05), and the introduction of 150 µl by 24.25% (P≤0.001) relative to the control.

Based on the data obtained, it can be said that the introduction of oak bark extract results in an increase in the digestibility coefficient of the feed. However, the inversely proportional relationship between the dose of EKD and the final percentage of digestibility should be noted: the higher the dose of EKD, the lower the digestibility, and, conversely, as the dosage decreases, the average percentage of digested feed substrate increases.

4. Discussion
The study of the therapeutic properties derived from natural products, in particular from plant extracts, has increased worldwide. [sixteen].

Treatment with herbal preparations provides some advantages compared with the use of chemicals [17,18].

This may be due to the fact that herbal preparations are a composition of various therapeutic or prophylactic compounds or components that can provide a pronounced activity in the prevention and treatment of diseases than a separate chemical substance [19].

The main recommended parameter when used in the feeding of plant extracts is safety and non-toxicity to the body. There are various ways to determine the toxicity of extracts and their components, most often using the in vitro method, for example, the method of collective behavior of C. Violaceum bacteria, characterized by the synthesis of the blue-violet pigment violacein [20], a common method is research on recombinant luminescent E. coli strains, allowing to obtain information on the biological activity of test substances in real time [21].

As follows from our data, aqueous extract of oak bark was characterized by the absence of toxic effect on the E.coli K12 TG1 model, as an independent component, and in combination with cicatricial fluid, at the same time, studies of alcohol extracts on C. Violaceum bacteria showed a more pronounced activity that manifested itself in an increase in the area of growth inhibition [22]. It is possible that the
development of luminescence induction is associated with the influence of some one or several components in the cicatricial fluid, which act as a nutrient substrate for bacterial cells, which has accelerated metabolic processes. It was revealed that oak bark extract on MPA nutrient medium suppresses the number of birds’ intestinal microflora [23].

According to a number of researchers, the use of plant extracts in animal husbandry, in particular, the use of their biologically active substances is one of the most significant areas in animal husbandry [24-26].

When using oak bark extract as an additive, the digestibility of the fodder substrate increased after 3 and 6 hours of exposure from 3.92% (P≤0.001) and 24.25% (P≤0.001) relative to the control. This may be due to the influence of small molecules contained in the extract, which have a total effect on the system of quorum feelings of pathogenic microorganisms, thereby increasing the overall percentage of digestibility [27].

Oak bark contains in its composition tannin, quercetin [28], which improves the use of feed by ruminant animals, mainly reducing the degradation of protein in the rumen, thereby contributing to greater availability of essential amino acids [29], due to which there is an increase in feed digestibility.

5. Conclusion
The introduction of bark extract in vitro gives an increase in the total percentage of digestibility of the feed substrate to 24.25% (P≤0.001) relative to the control. As part of further research, the results obtained will be used in in vivo experiments.

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