Comparative evaluation of the effect of the Quercus cortex extract and biologically active substances of plant origin on health and scar digestion

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Abstract. The paper studies effects of the Quercus cortex water extract (group II) and synthesized biologically active substances of the Quercus cortex extract (group III) on the dry matter digestibility, hematological parameters and the elemental composition of the scar fluid. It was identified that additives have a dose-dependent effect on the dry matter digestibility. They improve digestibility by 12.46% (P≤0.001) (group II) and 17.68% (group III). Among the hematological parameters, the number of lymphocytes increased by 34.07% (group II) and by 44.74% (group III); the hemoglobin concentration increased by 5.11% (group III). The serum iron decreased by 23.26% (P≤0.05) (group II) increased by 7.29% (group III). Experimental additives influence the microelement composition of the ruminal fluid reducing the concentration of Fe, CoCr, Ni and increasing the concentration of Mn, Cu, Zn values. The results obtained require further research.

1. Introduction

Currently, in order to correct the natural resistance and normalize metabolism in cattle, biological active substances of plant origin whose valuable components are well absorbed are used. In scientific and folk medicine preparations from aqueous plant extracts of larch wood, Siberian cedar and oak [1–2] are used.

Scientists found that biologically active substances have a positive effect on the mucous membrane of the digestive tract: they have an astringent effect similar to the tannic one, and contribute to the formation of a layer that reduces irritation of the mucous membrane [3].

When screening twenty medicinal plants used in medicine, a significant ability to inhibit the sensory quorum system of wild and mutant C. Violaceum strains was found in Quercus cortex extract, Betula verucosa buds and Eucalyptus viminalis leaves [4]. The content of biologically active systems in plants varies depending on various factors: parts, harvest seasons and geographic location, methods for producing additives [5]. Scientists conduct research on plant extracts, then carry out experiments adding isolated compounds of biologically active substances [6].

In addition, there is little information about the identification of compounds present in the additives, since most of the additives are complex extracts [7]. Therefore, it is recommended to identify chemical components in plant extracts in order to understand their effect on the elemental status of animals [8–9].
Thus, the aim of the article is to study the effect of an oak bark extract and biologically active substances of plant origin on health and digestion processes in ruminants, including morpho-biochemical blood parameters, digestibility, and the elemental composition of ruminal fluid.

2. Materials and methods

Two types of additives were studied: aqueous extract *Quercus cortex* and synthesized biologically active substances from the Quercus cortex extract.

There were two stages: the “invitro” stage (dry matter digestibility was determined by adding the aqueous extract *Quercus cortex* (1:10) at doses of 2.6; 3.3; 4.2; 4.7 mg/ml of the cicatricial fluid and synthesized biologically active substances (BAS EQC) at the same doses. Wheat bran (WB) was used as feed. Oak bark is a reddish-brown powder with specific odor, soluble in water (JSC "Krasnogorskleksredstva", Krasnogorsk, Russia).

The aqueous extract of oak bark was obtained by mixing 20 g oak bark with water in a volume of 200 ml, boiling over the water bath (30 min), filtrating, pressing and centrifuging for 15 minutes at 2000 rpm. The total volume of liquid was 200 ml. The composition was determined by a high-performance liquid chromatography on a gas chromatograph using a GQCMS 2010 Plus mass-selective detector (Shimadzu, Japan). When processing the results, we used GCMS Solutions, GCMS Post Run Analysis, to identify the compounds - a set of spectra CAS, NIST08, Mainlib, Wiley9 and DD2012 Lib. The aqueous extract included 36 active substances. Among them, the highest QS activity was found in 6 compounds: coniferyl alcohol, anti-arol, propyl resorcin, vanillin, coumarin, scopoletin. Based on the quantitative phytochemical analysis results, a drug consisting of biologically active substances of oak bark extract (*Quercus cortex*) was produced [10].

Ruminal fluid was taken through the rumen fistula. The studies were carried out using the “Artificial rumen KPL 01” according to the method by V. Lampeter modified by G.I. Levakhin, A.G. Mescheryakova [11].

500 g wheat bran dried to constant weight was mixed with the preparations (EQC and BAS EQC), placed in bags made of polyamide fabric. The bags were fixed on a platen placed in an artificial rumen device and a thermostat (TS-1/80 SPU, Smolensk SKTB SPU OJSC) at t = 39°C for 48 hours. The samples were washed under running water and placed in a solution of pepsin in the “artificial rumen” and in the thermostat for 24 hours. Digestibility of the dry matter of wheat bran was determined by the difference in the mass of the sample and the bag after the two-stage incubation and drying to constant weight at a temperature of 60 °C.

At the in vivo stage, studies were carried out on bulls of the red steppe breed aged 13 months. Animals were randomly divided into 3 groups (n = 15): one control and two experimental ones. For five days, in mornings, animals of the experimental groups were fed: I – control (without additives), II – with *Quercus cortex* 1: 10 (0.64 ml/kg per body weight), III – with synthesized biologically active substances obtained from Quercus cortex (0.81 ml/kg per body weight). To obtain the rumen fluid, a fistula was inserted. Samples (300 ml) were obtained before feeding, 3 and 6 hours after feeding. The dosage was chosen due to previously conducted invitro studies in per body weight.

Analysis of the elemental composition of rumen fluid involved determination of the concentration of trace elements: As, B, Co, Cr, Cu, Fe, I, Li, Mn, Ni, Se, Si, V, Zn using inductively coupled plasma mass spectrometry (MS-ICP) and atomic emission spectrometry with inductively coupled plasma (AES-ICP) on a Nexion 300D quadrupole mass spectrometer (“PerkinElmer” USA) and Optima2000 DV atomic emission spectrometer, (“PerkinElmer” USA). For ashing, the Multiwave 3000 microwave decomposition system (AntonPaar, Austria) was used.

In order to assess the health state and the metabolic rate, morpho-biochemical blood tests were conducted at the end of the accounting period. Blood sampling was carried out from the tail vein 2-3 hours before feeding; for hematological studies, vacuum tubes with EDTA-K3 were used; for biochemical tests, vacuum tubes with a clotting activator were used. The morphological blood parameters were evaluated using a URIT-2900 VetPlus automatic hematology analyzer (URIT Medical Electronic Co., Ltd. China). The biochemical blood serum composition was determined using an
automated biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd., China) and commercial biochemical kits for veterinary medicine (DIACON-DS, Russia; Randox Laboratories Ltd, United Kingdom).

Data were 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). The significance of group differences was estimated using Student’s t-test with p≤0.05.

3. Invitro study results

Invitro studies identified that dry matter digestibility increases due to the addition of biologically active substances *Quercus cortex* and an oak bark extract at various doses (Table 1).

| Group                  | Concentration (mg/ml) | Digestibility (%) |
|------------------------|-----------------------|-------------------|
| control (WB)           | -                     | 71.40 ± 0.53      |
| WB + E QC              | 2.6                   | 75.27 ± 0.55 ***  |
|                        | 3.3                   | 80.30 ± 0.87 ***  |
|                        | 4.2                   | 77.93 ± 0.58 ***  |
|                        | 4.7                   | 73.37 ± 0.41     |
| WB + BAS E QC          | 2.6                   | 87.27 ± 0.67 ***  |
|                        | 3.3                   | 79.00 ± 0.55 ***  |
|                        | 4.2                   | 84.03 ± 0.52      |
|                        | 4.7                   | 80.10 ± 0.55 ***  |

Note: wheat bran, * - P ≤ 0.05; ** - P≤0.01; *** - P≤0.001.

When making adding the Quercus cortex extract at the minimum dose, dry matter digestibility exceeded the control one by 5.42% (P <0.01). An increase in the dose to 3.3 mg/ml increased digestibility by 12.46% (P≤0.001). A further increase decreased digestibility.

Increasing concentrations of biologically active substances increase digestibility from 8.22% (P≤0.001) to 17.68%.

When comparing two factors, it is clear that biologically active substances increase digestibility more as compared to the aqueous extract *Quercus cortex*.

**Invivo study results.**

**Morpho-biochemical blood parameters.**

The Quercus cortex extract (group II) decreased the number of granulocytes and platelets by 28.94 and 3.41% (P≤0.05), respectively. The number of lymphocytes and erythrocytes increased by 34.07% and 0.34%, respectively.

Biologically active substances (group III) increased the hemoglobin content by 5.11% and decreased platelets by 48.3% (P<0.01) (Table 2). The Quercus cortex (group II) increased activity of ALT by 8.10%, and biologically active substances (group III) reduced activity of the enzyme by 18.81% (P≤0.01) (Table 2).

In experimental group III, the calcium content increased by 3.00% (P ≤ 0.05). In group II, the iron content decreased by 23.26% (P≤0.05), while in group III, it increases by 7.29%. The blood phosphorus content increased by 268.4% (group II), and by 105.26% (group III) (Table 3).
Table 2. Morphological blood parameters of animals at the end of the accounting period (M±m, n=15)

| Indicator                  | Group I (without introduction) | Group II (E QC ) | Group III (BAS E QC ) |
|----------------------------|--------------------------------|------------------|-----------------------|
| leukocytes, 10^9 / l       | 12.00 ± 1.947                  | 8.13 ± 0.146     | 13.17 ± 1.328         |
| lymphocytes, %             | 35.63 ± 2.264                  | 47.77 ± 2.344    | 51.57 ± 2.153         |
| monocytes, %               | 27.73 ± 1.782                  | 26.20 ± 0.923    | 25.03 ± 1.442         |
| granulocytes, %            | 36.63 ± 1.783                  | 26.03 ± 1.268    | 23.40 ± 1.424         |
| red blood cells, 10^{12} / l | 5.89 ± 0.396                  | 5.91 ± 0.152     | 5.56 ± 0.323          |
| hematocrit, %              | 84.67 ± 1.856                  | 86.00 ± 1.155    | 89.00 ± 3.055         |
| platelets, 10^9 / l        | 185.67 ± 6.965                 | 179.33 ± 7.89    | 96.00 ± 4.933         |

Note: * - P≤0.05; ** - P≤0.01

Table 3. Biochemical blood parameters of animals at the end of the accounting period (M±m, n = 15)

| Indicator                  | Group I (WB) | Group II (E QC ) | Group III (BAS E QC ) |
|----------------------------|--------------|------------------|-----------------------|
| ALaT, E/l                  | 41.10 ± 0.265 | 44.43 ± 2.069    | 33.37 ± 2.258         |
| ACaT, E/l                  | 133.37 ± 2.211 | 112.3 ± 4.708    | 100.23 ± 2.138        |
| Alkaline phosphatase, u/l  | 92.33 ± 4.518 | 47.67 ± 4.667    | 71.67 ± 3.212         |
| GGT, u/l                   | 7.67 ± 0.882  | 7.33 ± 1.202     | 7.33 ± 1.856          |
| Calcium, µmol/l            | 1.33 ± 0.009  | 1.36 ± 0.009     | 1.37 ± 0.010          |
| Uric acid, µmol/l          | 28.00 ± 3.430 | 25.73 ± 3.369    | 23.00 ± 2.629         |
| Creatinine, µmol/l         | 134.50 ± 3.675 | 114.80 ± 4.970   | 113.13 ± 1.419        |
| Glucose, µmol/l            | 3.44 ± 0.164  | 3.48 ± 0.290     | 3.83 ± 0.114          |
| Total protein, g/l         | 84.46 ± 1.281 | 79.80 ± 1.853    | 73.05 ± 0.920         |
| Bilirubin, µmol /l         | 0.60 ± 0.000  | 0.51 ± 0.095     | 0.60 ± 0.051          |
| Bilirubin direct, µmol/l   | 1.01 ± 0.035  | 0.89 ± 0.035     | 0.81 ± 0.051          |
| Cholesterol, µmol/l        | 3.15 ± 0.119  | 3.19 ± 0.271     | 3.36 ± 0.310          |
| Urea, µmol/l               | 5.20 ± 0.611  | 2.57 ± 0.067     | 3.07 ± 0.406          |
| Iron, µmol/l               | 28.80 ± 2.023 | 22.10 ± 1.501    | 30.90 ± 4.670         |
| Phosphorus, µmol/l         | 0.19 ± 0.059  | 0.70 ± 0.075     | 0.39 ± 0.076          |

Note: * - P≤0.05; ** - P≤0.01; *** - P≤0.001

In experimental groups, the urea content decreased by 50.58% (P≤0.01) and 40.96% (P≤0.05) and the creatinine content - by 14.65% (P≤0.01) and 15.89% (P≤0.01), respectively.

Biologically active substances (group III) decreased the total protein content by 19.80% (P≤0.01) and 13.51% (P≤0.001), respectively. The remaining biochemical parameters were close to the control values.

The elemental composition of rumen fluid. The number of trace elements changed. 3 hours after the addition of the Quercus cortex extract (group II), the As concentration decreased by 50% (P≤0.001), Ni - by 26.92% (P≤0.001), and Fe - by 32.81% (P≤0.01). Cu decreased by 66.67% (P≤0.001), Mn - by 28.13% (P≤0.001), Zn - by 111.46% (P≤0.05) (Fig. 1).
Figure 1. The difference in the concentration of elements in the cicatricial fluid three hours after the addition of the *Quercus cortex* extract (group II) compared with the control group,%

Three hours after the addition of biologically active substances of the *Quercus cortex* extract (group III), As decreased by 50.0% (P≤0.001), Fe – by 41.34% (P≤0.001), and Cu increased by 66.7% (P≤0.001), Mn – by 41.19% (P≤0.001), and Zn – by 93.63% (P≤0.01) (Fig. 2).

Figure 2. The difference in the concentration of elements in the cicatricial fluid three hours after the addition of biologically active substances of the *Quercus cortex* extract (group III) compared with the control group,%

Six hours after the addition of EQC (group II), the concentration of As decreased by 60%, Cr - by 40.91% (P≤0.001), and Fe - by 50% (P≤0.001). In the rumen fluid, the concentration of Cu increased by 21.21% (P≤0.001%), Mn - by 12.62% (P≤0.01) (Fig. 3).
Figure 3. The difference in the concentration of elements in the cicatricial fluid six hours after the addition of Quercus cortex extract (group II) compared with the control group, %

Biologically active substances of the Quercus cortex extract (group III) increased the concentration of Cu by 24.24% (P≤0.05). Cu increased by 19.44%, Mn – by 14.07%, S – by 38.18%. Cr and Fe decreased by 45.94% (P≤0.001) and 56.01% (P≤0.001) (Fig. 4).

Figure 4. The difference in the concentration of elements in the rumen fluid six hours after the addition of biologically active substances of the Quercus cortex extract (group III) as compared with the control group,%

In the experimental groups, the content of trace elements in the rumen fluid changed. Their higher content was observed 6 hours after addition of the extract due to the accumulation of chemicals during digestion.

4. Discussion
The Quercus cortex extract of biologically active substances increased digestibility by 12.46% (P≤0.001) and 17.68%; Quercus cortex as a source of quercetin has antioxidant and anti-inflammatory effects increasing digestibility of nutrients in the rumen [12].

Quercus cortex as a source of tannin has no negative effect on fermentation. It has a positive effect on dry matter digestibility, energy metabolism and the use of protein in the rumen [13].
Inhibiting properties of medicinal plants and their extracts are associated with the participation of phenols and polyphenols (flavonoids) in reactions with radicals that accompany some diseases [14].

*Quercus cortex* and biologically active substances of plant origin changed morphological and biochemical blood parameters; the number of leukocytes, lymphocytes, monocytes, granulocytes increased [15] due to an increase in immunological activity and phagocytosis [16].

An increase in the content of leukocytes in group III is consistent with previous studies [17] performed using the thyme extract, where there was no significant increase in the level of leukocytes, but immunological reactions improved.

Feeding broiler chickens with *Quercus cortex* + artificially synthesized substances has a positive effect on the immunomodulating state and antioxidant activity, increases the content of in-lysine, serum superoxide dismutase and catalase [18].

The data obtained are similar to the data in [19], where a decrease in plasma iron was observed when feeding with *Grape seed extracts*.

Protein plays a leading role in complex biochemical processes; its content in the blood plasma indicates the physiological well-being of the body [20]. The concentration of total protein in the blood of calves increases as an adaptive-mobilization response. In the blood of experimental animals, the level of total protein is lower than the control value which indicates higher metabolism in the animals of the experimental group [21].

50 g of dry matter of the oak bark extract added into the feed increased calcium and sodium twice; phosphorus and magnesium - 1.7 and 1.8 times [22].

The *Quercus cortex* extract increased the Mn concentration due to its high concentration in the extract and its ability to form weak systems with chemical elements in the gastrointestinal tract [23].

In the experimental groups, the iron content decreased. It is in compliance with the report of a decrease in the content of zinc and copper in the liver of monogastric animals fed with plant products (extracts from grape marc) containing polyphenolic substances [24].

5. Conclusion

It was established that the *Quercus cortex* extract and synthesized biologically active substances of the *Quercus cortex* extract in cattle feeding have a dose-dependent effect on dry matter digestibility. Digestibility increased by 12.46% (P≤0.001) (group II) and 17.68% (group III). The number of lymphocytes increased by 34.07% (group II) and 44.74% (group III); the hemoglobin concentration increased by 5.11% (group III). Serum iron decreased by 23.26% (P≤0.05) (group II) and increased by 7.29% (group III). Experimental additives had an effect on the microelement composition of the rumen fluid reducing the concentration of Fe, Co, Cr, Ni and increasing the concentration of Mn, Cu, Zn. The results require further research.

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