Stimulation of ruminal digestion of young cattle with oak bark extract (Quercus cortex)

K N Atlanderova1, A M Makaeva1, E A Sizova1,2, G K Duskaev1

1 Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, 29, 9 Yanvarya str., Orenburg, 460000, Russia
2 Orenburg State University, 13, Pobedy ave., Orenburg, 460018, Russia

E-mail: atlander-kn@mail.ru

Abstract. The significance of rumen for the body of ruminants determines the prospects for improving the efficiency of ruminal digestion, which can be achieved through the use of probiotics, antimicrobial metabolites and other drugs. Recently, plant extracts of medicinal plants have been actively used, for example, oak bark extract (Quercus cortex). Based on the results of recent studies, antiquorum substances are one of the extract components. According to the results of in vitro study, it was established that addition of oak bark extract (OBE) in various concentrations into ruminal fluid (RF) affects enzymatic activity and is accompanied by a change in feed digestibility from 2.42% (P <0.05) to 7.13% (P <0.01). The greatest digestibility is achieved after the introduction of OBE in a dosage of 3.3 mg/ml. The use of EKD leads to an increase in the pH of the ruminal fluid to 6.7-6.8 and stimulation of enzymatic activity of microorganisms, therefore, leads to an increase in digestibility and absorption of nutrients.

1. Introduction
The success of cattle feeding largely depends on the vital activity of ruminal microbiome, which provides the host organism with minerals, energy, protein, and many other significant substances [1]. Stimulation of the microbiological system of rumen is a way to improve the efficiency of feed use through the use of biologically active substances that affect the development of ruminal microflora, including extracts of phytobiotics [2], amino acids [3], micro- and macroelements in various forms [4-6], enzymes [7, 8]. Biologically active substances of plant origin, used to correct cicatricial digestion, account for one third of the total list of these complexes used to treat the diseases of digestive organs [9]. The latter include drugs produced from Quercus cortex. The list of biologically active substances of Quercus cortex is significant and includes tannins, coumarins, catechins, starch, sugars, vitamins, tannin and quercetin, etc.

OBE, introduced into the stomach, enhances its motility, increases digestibility, reduces the enzymatic activity and acidity of the gastric contents, and also slows down the absorption of the gastric mucosa. The compounds in the content of OBE affect the functional state of digestive organs and heal ulcerative defects [10].
It was established that the complex of plant substances in the composition of OBE showed a pronounced ability to suppress the sense of quorum in bacteria. Among 36 identified components, 7 compounds had the highest activity, their anti-quorum effect increased in the series: 4-(3-hydroxy-1-pro-phenyl)-2-methoxyphenol>3.4.5-trimethoxyphenol>7-hydroxy-6-methoxy-2H-1-benzopyran-2-one>2H-1-benzopyranon-2>4-hydroxy-3-methoxybenzaldehyde>1.2.3-trihydroxybenzene>propyl-1.3-benzene diol [11].

This type of biological activity of extracts of phytogenic compounds is determined by the combined effect of the small molecules present in them, which have a total effect on the system of quorum sense of pathogenic microorganisms [12, 13]. The aim of our research is to study the effects of OBE on ruminal digestion and digestibility of dry matter in vitro.

2. Materials and methods

Studies were conducted using RF, obtained from the bulls of the Red Steppe Breed of the dairy productivity direction at the age of 13 months. Ruminal fluid was collected using chronic fistula. All experimental procedures were approved by the Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences and were performed according to the guidelines set forth in the Guide for the Use and Care of Laboratory Animals [14].

Studies were conducted using the mobile nylon bag technique: in vitro - using the "artificial rumen KPL 01", 48-hour exposure.

OBE was used as a test substance, and wheat bran (WB) in natural form was used as feed. OBE was obtained mixing 50 g of substance with water in a volume of 500 ml and boiling in a water bath (LOIP LB-140, Sibagropribor, Russia) (30 min), followed by filtration and squeezing through a filtering component and bringing the total volume of boiled liquid water to 500 ml, which is then centrifuged. OBE was injected into nylon bags of food in various concentrations (2.6; 3.3; 4.2; 4.7; 5.0 mg / ml), then laid in an artificial rumen.

The determination of chemical substances contained in extract was carried out on a gas chromatograph with a GQCMS 2010 Plus mass-selective detector (Shimadzu, Japan) on an HP-5MS column. To process the results, we used GCMS Solutions software, GCMS PostRun Analysis. The quantitative presence of some identified components was assessed according to the ratio (%) of peak area to extract area [15].

Digestibility of dry matter of feed substances was analyzed according to the method of Dr. V. Lampeter modified by G.I. Levakhin, A.G. Meshcheryakov [16]. The acidity of ruminal environment was measured using a pH meter (pH-150 MI, OKB SPECTR LLC, Russia).

In vitro digestibility of dry matter of feed was determined from the difference of the mass of feed sample together with the bag and after two-stage incubation and drying to constant weight at a temperature of +60 °C using the following formula:

\[ K = \left( \frac{A-B}{C} \right) \times 100\% \]

where

- \( K \) - coefficient of digestibility of dry matter of feed (%);
- \( A \) - the initial mass of feed sample, together with the bag (g);
- \( B \) is the mass of feed sample together with the bag after digestion (g);
- \( C \) - the initial mass of feed sample without the mass of bag (g).

Based on in vitro results, the optimal dosage was determined for further use.

Further study of the parameters of ruminal digestion was carried out under in situ conditions after 3 and 6-hour exposure. Two groups of bulls were formed (n = 5): nylon bags with samples of dried food (5 gr.) is placed in the rumen (I group without additives), group II - feed with OBE in a dose of 3.3 mg for direct study of drug in the body of cattle.

The amount of microbial mass was determined by the method of differential centrifugation (MiniSpin centrifuge, Merck KGaA, Germany) and further drying to constant weight. The calculation of ciliates was...
carried out by a microscopic method (Levenhuk binocular microscope, Levenhuk company, Russia) in a Goryaev chamber.

Cellulolytic activity was evaluated in accordance with the method [17]. The percentage content of cellulolytic activity was determined by the difference of weight of the cellophane strips before and after incubation.

Amylolytic activity was determined evaluating the starch cleavage by microbial amylase, with the fixation of the processes on the spectrophotometer SF-2000 and was calculated according to the formula:

\[ x = (A - B) \times 20, \]

where \( x \) is the amount of starch cleaved by 1 ml of paunch manure for 1 h, mg;

A - the amount of starch in the solution before incubation, mg;

B is the amount of starch after incubation, mg;

20 - the coefficient of recalculation per 1 ml of paunch manure.

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 group differences was estimated using Student’s t-test with \( p \leq 0.05 \) considered as significant.

3. Results and discussion

3.1 In vitro Results

In vitro data showed that the digestibility of dry matter of feed additives increases with the addition of OBE in various dosages (Fig. 1).

![Figure 1. The digestibility of feed dry matter after OBE in various concentrations, %](image)

When minimum dose of OBE was added, dry matter digestibility index was by 6.03\% more (\( p < 0.01 \)) compared with the control. As the dosage was increased to 3.3 mg/ml, digestibility increased with a difference of 7.13\% with control (\( p < 0.01 \)). The lowest efficiency of dry matter digestibility is marked against the background of OBE in a dosage of 4.7 mg / ml.

Fluctuations in the concentration of hydrogen ions were in the range of 6.12–6.82 pH at different concentrations. Analyzing data (Table 1), it can be noted that the lowest pH was observed in the control group. In all other experimental groups, pH values shifted closer to the alkaline medium.
Table 1. Hydrogen index (pH) of ruminal fluid after addition of OBE in various concentrations

| Name          | Concentration of OBE, mg/ml | pH       |
|---------------|----------------------------|----------|
| Control       | -                          | 6.12±1.01|
| WB+OBE        | 5.0                        | 6.61±0.15|
| WB+OBE        | 4.7                        | 6.67±0.19|
| WB+OBE        | 4.2                        | 6.82±0.09|
| WB+OBE        | 3.3                        | 6.81±0.20|
| WB+OBE        | 2.6                        | 6.77±0.06|

3.2 Results in situ
So, 3 hours after feeding, a decrease in the number of bacteria in the experimental group was observed in relation to the control, which may be a manifestation of the bactericidal effect of OBE (Table 2).

Table 2. The microbiota composition of ruminal fluid after OBE use

| Exposure | Group          | Name | bacteria, g/100 ml | ciliates, thou./ml |
|----------|----------------|------|-------------------|-------------------|
| Before feeding | -              |      | 0.30±0.10         | 628.25±14.11      |
| 3 hours after   | Control        |      | 0.41±0.13         | 789.05±11.52      |
|                 | Experimental   |      | 0.18±0.11         | 883.10±16.56**    |
| 6 hours after   | Control        |      | 0.32±0.10         | 486.90±8.92       |
|                 | Experimental   |      | 1.21±0.12**       | 685.43±10.21***   |

Significant difference in relation to control; * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.

The presence of more than 500 thousand ciliates in the rumen indicates a normal and effective course of enzymatic processes [18]. OBE in its composition has some amount of easily hydrolyzed carbohydrates, thereby contributing to an increase in the number of ciliates. The largest number of ciliates was observed 3 hours after feeding.

Celluloseolytic microorganisms are among the most important symbionts of ruminants, in terms of the digestibility of vegetable feed. This group of microorganisms substantially complements the enzymatic equipment of ruminants and allows them to extract energy from feeds inaccessible to most mammals. We found that in the experimental bulls, the enzymatic activity of the scar fluid was higher compared with the control animals. Thus, in the Experimental cellulolytic activity, the group exceeded the control group by 14.2% after 3 hours and by 26.2% (P≤0.01) after 6 hours. An increase in exposure up to 6 hours resulted in an increase in amylolytic activity in the experimental group by 7.6% (P≤0.001), compared with the control (Table 3).

Table 3. Enzymatic activity of ruminal fluid after use of OBE

| Exposure | Group          | Activity             |
|----------|----------------|----------------------|
|          |                | Celluloseolytic, %   | Amylolytic, %       |
| Before feeding | -              | 6.15±0.13            | 67.27±0.67          |
| 3 hours after   | Control        | 14.43±0.86           | 68.32±0.58          |
|                 | Experimental   | 16.48±0.75           | 71.85±0.68**        |
| 6 hours after   | Control        | 10.15±0.34           | 62.11±0.51          |
|                 | Experimental   | 12.81±0.59**         | 66.83±0.41***       |

Significant difference in relation to control; * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.
4. Conclusion

OBE has antimicrobial activity, which was previously explained by the presence of astringent and anti-inflammatory substances, including some tannins, gallic and ellagic acid, and quercetin. Meanwhile, it has recently become clear that substances that suppress the sensitivity of bacteria to a quorum (QS) are equally important components of OBE that have antimicrobial effects. Under the action of “small molecules” contained in OBE, some pathogenic groups of microorganisms are inhibited [11], which is directly reflected in the quantitative composition of bacteria of the experimental group.

Oak bark is a source of tannin, which has not negative effect on fermentation in the rumen of cattle [19], and has a positive effect on the digestibility of dry matter, energy exchange and the use of protein in rumen [20]. In a dosage of 3.3 mg / ml it has the most significant effect on the digestibility of the feed substrate. In our opinion, this effect is associated with an increase in microbial enzymatic activity. At the same time, OBE, being a source of quercetin, rutin, has antioxidant and anti-inflammatory effects [23-25], promotes an increase in milk [26], meat [27] and egg [28] productivity.

It is important that the use of phytogenic additives is assessed as a means of increasing productivity of animals. The effect of tannins apparently consists not only of its direct participation in digestion by binding dietary proteins, but also of the effect on microbiota of the gastrointestinal tract [29-31]. The decrease in decomposition of crude protein by symbiotic microorganisms against the background of the use of tannins leads to a decrease in nitrogen losses in the form of ammonia in rumen [32].

OBE affects ruminal microflora, thereby contributing to the correction of ruminal digestion [33]. In particular, the environment where ruminal microorganisms is important. Therefore, after the introduction of OBE there was an increase in pH. The reaction of rumen environment (pH) is associated with the flow of enzymatic processes and the formation of metabolites, therefore, from the point of view of the characteristic of rumen digestion, this indicator is significant [34]. Fluctuations in the pH of rumen depend mainly on the level of organic acids, bicarbonates and phosphates in it [35]. Changes in microbiological processes under the influence of oak bark extract are confirmed by the pH dynamics. As follows from the obtained data, this indicator turned out to be higher than in the control in all experimental groups, it is explained by the presence of tannins, which increase the pH [29]. Alkalization of ruminal contents is possible due to the increase in ammonia production, which is associated with an improvement in the digestibility of rations [36-38].

The results require further research to assess the effect of herbal extracts on microbiota and parameters of bovine rumen fermentation.

5. Conclusion

In vitro studies have shown that addition of OBE in various concentrations increase the digestibility of feed substrate by an amount from 2.42% (P <0.05) to 7.13% (P <0.01). The highest digestibility of feed is achieved when OBE is added at a dosage of 3.3 mg/ml, and the pH of ruminal fluid is shifted toward the neutral one. An in situ experiment showed that the use of OBE causes the stimulation of amylolytic and cellulolytic activities of ruminal fluid microbiota and an increase in the number of ciliates, against the decrease in the number of bacteria. The quantitative and qualitative composition of biomass depended on the exposure time.

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