Increasing the efficiency of beef production by means of correcting cicatricial digestion with a mineral complex and plant extract

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Abstract. The study presents the results of evaluating the effect of oak bark extract (OBE) and FeCo alloy mineral complex (MC) on digestion processes in the rumen of cattle, in particular, digestibility, hydrogen index and microbiome. The use of FeCo alloy mineral complex with OBE leads to an increase in digestibility of dry matter of the feed by 12.0 % as compared to the control. Changes in the hydrogen ions concentration were in the range of 7.0–8.10 pH. It was revealed during the experiment that both individual and joint introduction of OBE and FeCo mineral complex affects quantitative content of ciliates and bacteria. 6 hours after feeding, the number of ciliates increased with introduction of OBE by 20.4 % relative to the control and protozoa mass with the addition of FeCo mineral complex increased by 111 % (p≤0.01). The data obtained when introducing combination of OBE and FeCo alloy mineral complex indicate an increase in ruminal digestion through the synergistic effect of the mineral complex and FeCo alloy. The studied substances can be used in the industrial production of animal feed.

1. Introduction

The current trend in animal feeding is a diet with “natural” substances since the use of certain additives, such as antibiotics, is limited at the legislative level due to development of bacterial resistance [1]. This has created the prerequisites for the search for safe alternatives that may be applicable in animal breeding. Currently, probiotics, prebiotics, herbs, minerals and plant substances are considered good substitutes for feed antibiotics [2]. Some plant extracts are biologically active substances with a high potential of antioxidant properties, immunotropic and growth-promoting effects [3]. Known as phytobiotics, they are used in animal feeding, as antimicrobial, anti-inflammatory, antioxidant and antiparasitic agents [4]. It is known that plant extracts has been used since ancient times to prevent or treat animal diseases due to the availability, ease of use and lack of side effects. One of the plants studied is Quercus robur. It was found that its extract has antioxidant, antifungal, antibacterial and antitumor activity [5]. In addition, being an inhibitor of the quorum sensitivity system (QS) in bacteria, it affects reorganization of the gastrointestinal tract bacterial flora and, as a result, the performance of farm animals [6].

Intensive development of delivery substances based on ultrafine mineral complexes will lead to the appearance of preparations with improved pharmacological and pharmacokinetic properties for use in animal feeding [7–10].
Interaction of plant extracts containing tannins with organic compounds or trace elements is of interest and may potentially influence nutrients digestibility. It is obviously that thorough research of tannins use in complexes with mineral elements in animal breeding will assist in understanding the role of tannin chemistry in interacting with the animal body. It is established that plant extracts associated with mineral complexes alloys have advantages over conventional forms [11]. When associated with mineral complexes they have increased elimination half-life \textit{in vivo}.

2. Materials and methods
The purpose of the study was to evaluate the effect of OBE and FeCo mineral complex on the digestibility of feed substrate dry matter and rumen microflora \textit{in situ}. The studies were conducted on bulls of the red steppe breed (n = 24) aged 14 months with chronic rumen fistula. Collection of ruminal fluid was performed in 3 and 6 hours after feeding.

Animal treatment and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 08/12/1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)”. During the study there were made efforts to minimize animal suffering and reduce the number of samples used.

Oak bark extract was used in the experiment. It was obtained by mixing 20 g of dry matter with distilled water in a volume of 200 ml, 30 minutes exposure in a boiling water bath followed by filtration. The filtrate was brought to a volume of 200 ml with water. Next, the extract was centrifuged on an OPn8 medical centrifuge (PO Box V-2331, Russia).

Mineral complexes of FeCo alloy were used in the experiment. Preparation of a mineral complexes suspension: FeCo alloy was weighed on a laboratory electronic balance MB 210-A (Sartogosm CJSC, Russia), exact weights were dispersed in an isotonic solution by ultrasonic treatment for 30 minutes at a frequency of 35 kHz (power – 300(450) W, oscillation amplitude is 10 $\mu$m) on an ultrasonic disperser UZDN-2T (NPP Akademprimorib, Russia).

To research the properties of feed additives using \textit{in situ} method, 5.0 g of a dried feed sample mixed with experimental additives was used: Group I (control, no additives), Group II – OBE at a dose of 3.3 mg/ml, Group III – OBE together with FeCo alloy mineral complex (dose 3.3 mg/ml + 0.75 mg/ml), Group IV – FeCo alloy mineral complex (0.75 mg/ml). They were placed in the nylon bags. Filled bags were immersed in the animal rumen through the fistulous opening for 3 and 6 hours. At the end of the period the bags were removed, dried at $t = 60^\circ$ in a drying cabinet SHS-80-01-SPU (Smolenskoye SKTB SPU, Russia) with subsequent determination of the dry matter digestibility in the rumen by difference in weight before and after drying.

The dosage was selected based on the previous studies \textit{in vitro} [12]. The amount of ruminal fluid’s microbial mass was determined by differential centrifugation on a MiniSpin centrifuge (MerckKGaA, Germany) and further drying to constant weight. The ciliates were counted in the Goryaev counting chamber using a Mikromed 1 microscope (Zdravtorg, Russia).

The hydrogen index in the ruminal fluid was determined using the pH analyzer ph-150MI (Measuring Equipment LLC, Russia) in accordance with the instructions.

The study was conducted on the base of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences as well as of the Core Facility Center, Federal Research Center.

Statistical analysis. Data are expressed as mean values \pm 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
\textit{In situ study results.}
Data analysis showed that the digestibility of the feed substrate dry matter in the in situ experiment
increases in 6 hours after feeding. Introduction of OBE increases digestibility of dry matter by 11.6 % compared with the control group. Introduction of FeCo alloy mineral complex increased digestibility relative to the control by 15.1 % (p≤0.05). The use of the FeCo alloy mineral complex and OBE composition has a greater effect on digestibility by increasing it by 12.0 % (p≤0.05) relative to the control (Figure 1).

**Figure 1.** Digestibility of feed substrate after 3 and 6 hours exposure, concentration expressed in mg/ml

Differences in the hydrogen ions concentration were in the range of 7.0–8.10 pH with various additives. Analyzing the data (Table 1), it can be noted that the lowest pH values were observed when using the FeCo alloy mineral complex and OBE composition during the experiment, the pH values in all other experimental groups turned out to be higher, the neutral rumen environment is favorable for the microorganisms growth.

**Table 1.** Ruminal fluid pH with introduction of experimental preparations after three, six hours of feeding

| Group         | Exposure, (h) | 3        | 6        |
|---------------|---------------|----------|----------|
| Control       | 7.9±0.39      | 8.06±0.40|          |
| OBE           | 7.07±0.08     | 6.77±0.03|          |
| OBE + FeCo    | 7.0±0.35      | 7.1±0.35 |          |
| FeCo          | 8.10±0.40     | 8.10±0.48|          |

Due to an increase of the exposure time of rumen digestion, the number of protozoa and bacteria changes compared to the control (Table 2).

**Table 2.** The effect of additives on the rumen microbiome content in cattle

| Exposure, (h) | Group       | Ciliates, thou./ml | Biomass, g/100 ml | Name     | Protozoa | Bacteria |
|---------------|-------------|--------------------|-------------------|----------|----------|----------|
| 3             | Control     | 511±35.8           | 1.29±0.01         |          | 0.31±0.01|          |
|               | OBE         | 657±12.1**         | 1.95±0.03**       |          | 0.15±0.01|          |
|               | FeCo        | 551±27.6*          | 2.2±0.03          |          | 0.14±0.04|          |
|               | OBE + FeCo  | 594±29.7*          | 1.43±0.02         |          | 0.17±0.02*|         |
| 6             | Control     | 500±25.8           | 0.9±0.03          |          | 0.29±0.01|          |
|               | OBE         | 602±18.1           | 1.6±0.06          |          | 0.10±0.001|         |
|               | FeCo        | 513±28.2           | 1.9±0.03**        |          | 0.13±0.03*|         |
|               | OBE + FeCo  | 530±26.9           | 0.93±0.03         |          | 0.12±0.03|          |

Significant difference in relation to control; * p ≤ 0.05, ** p ≤ 0.01
When OBE is introduced into the diet three hours after feeding, an increase in the ciliates concentration by 28.6 % (p ≤ 0.01) relative to the control is observed, introduction of the OBE and FeCo alloy mineral complex composition reduces the effect on ciliates and its concentration increases by 16.2 % (p ≤ 0.05) compared to the control.

The protozoa significantly increased with the introduction of OBE – by 51.2 % (p ≤ 0.01), after 3 hours of exposure, while 6-hour digestion revealed an increase in protozoa when using FeCo by 111 % (p ≤ 0.01).

4. Discussion

One of the most characteristic features of plant extracts is the tannins content which dominant feature is sorption activity able to attach metals through hydrogen bonds. The complexing properties of tannins are associated with the presence in its aromatic ring compounds of hydroxyl and/or carboxyl groups capable of forming ionic or donor – acceptor bonding with Fe$^{2+}$ cations [13].

The supply of preparation compounds with transport systems eliminates many of the shortcomings of developed and existing preparations – low solubility in water, rapid sorption or metabolism in the body, difficulty in crossing natural membranes and barriers [14].

According to a number of researchers, the use of plant extracts in animal breeding, in particular, use of their biologically active substances is one of the most significant areas. Literature sources contain articles on the ability of metal ions to chelate commercially available tannins [15]. However, there is little data on the complex formation of ionic metals with tannins obtained from plant extracts. In addition, medicinal plants are inhibitors of the Quorum Sensing System (QS) in bacteria [16]. Such inhibitors are found in oak bark extract.

When using OBE as an additive, the digestibility of the feed substrate increased to 11.6 % (p≤0.05) in 6 hours after feeding 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 Quorum Sensing System of pathogenic microorganisms, thereby increasing the overall percentage of digestibility [17].

Oak bark contains tannins, quercetin, which improve the use of feed by ruminants, reducing protein degradation in the rumen thereby contributing to its greater availability due to which there is an increase in feed digestibility. Introduction of OBE and FeCo alloy mineral complex composition increases digestibility up to 15.1 % (p≤0.05) probably due to the fact that cobalt affects fermentation parameters in the rumen and synthesis of microbial vitamin B$_{12}$ [18].

With introduction of grain feed into the diet, the fermentation nature in the rumen changes, the pH begins to decrease. The interaction of substances in the rumen with feed, as well as the microbiocenosis state depends on pH [19] and the feed additives affect the antioxidant ability [20]. Use of OBE and FeCo alloy mineral complex in feeding shifted the hydrogen index closer to the neutral medium, thereby ensuring the result of microflora growth.

The success of ruminal digestion directly depends on the vital activity of symbiotic microorganisms and protozoa [21]. Bacteria break down and digest fiber, which is of great importance for the nutrition of ruminants. Over time, a gradual decrease in the biomass of bacteria is observed. This tendency may be associated with the microorganisms activation to participate in the digestive processes in ruminants forestomach or with active adherence on the food coma surface [22].

5. Conclusion

Thus, the combined use of OBE and FeCo alloy mineral complex positively affects the digestive processes in the rumen of cattle. In particular, in situ conditions increase the feed substrate digestibility against the background of the numerical advantage of ciliates and protozoa.

At the same time, the data obtained requires further research, including evaluation of the results of metagenomic sequencing of the microbiota of the cattle ruminal digesta.

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