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Effect of *Artemisia absinthium* (*Asteraceae*) and Cobalt Supplementation on Rumen Bacterial Community in Cattle

V.A. Ryazanov[1], G.K. Duskaev[1], E.V. Sheida[1], Y.A. Khlopko[2], A.O. Plotnikov[2]

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ABSTRACT

Background: One of the key tasks of livestock development is to reduce the amount of feed needed for raising and fattening cattle, as well as increase the efficiency of feed use. A possible solution to these problems are plant-based feed additives containing various phytochemical compounds, both individually and together with metallic trace elements, to improve the functioning of the gastrointestinal tract of animals. The aim of the study was to assess the effect on the microbiota of the cattle rumen of the vegetable feed components *Artemisiae absinthil* (bitter plant *A. absinthium*) and $CoCl_2$ (cobalt II chloride).

Methods: Study the effect of the herb *Artemisia absinthium* (*A. absinthium*) and $CoCl_2$ (cobalt II chloride) on the microbiome of cattle rumen. The studies were conducted on a cannula animal model, *Bos taurus* (Kazakh white-headed cattle), after feeding an experimental feed additive, *A. absinthium*, either alone or in combination with $CoCl_2$. The experiment was carried out in four repetitions using a 4 × 4 Latin square. Animals in the I experimental group were additionally given *A. absinthium* at a dose of 2.0 g/kg of dry matter (DM); in the II experimental group, *A. absinthium* at a dose of 2.0 g/kgDM with additional $CoCl_2$ (1.5 mg/kgDM); and in the III experimental group, only $CoCl_2$ (1.5 mg/kgDM). The contents of the scar were collected for further analysis, which was performed by high-performance sequencing (NGS) of 16S rRNA gene ampicons.

Result: There was difference in the microbiome of the rumen of cattle between the control and experimental groups, both at the level of the phylum's and at the genus level. The dominant phylum in all groups were *Firmicutes* and *Bacteroidetes*. A study of the microbiome of the rumen revealed groups of microorganisms capable of producing carbohydrate-active enzymes (CAZyms) and destroying structural hydrocarbons. In the group using *A. absinthium*, a symbiotic link was established for the genus *Akkermansia* and the unclassified *Bacteroidales* linear regression (R^2= 0.93, p= 0.0435) and for the unclassified *Ruminococcaceae* and the genus *Akkermansia* (R^2= 0.75, p= 0.05).

Key words: Cattle, Feed additives, Microbiome, Rumen.

INTRODUCTION

Growing land populations, climate change (Bačėninaitė *et al.,* 2022 and Kaur *et al.,* 2017) require more precise approaches to livestock production (Lachenmeier, 2010 and Austen, 2021), in particular cattle farming. One way to intensify the growing and feeding of cattle is to use specialized feed or feed additives (Monteiro *et al.,* 2022 and Gill *et al.,* 2006), which can regulate physiological processes in the gastrointestinal tract of animals without indirectly affecting the microbiome (Thomas *et al.,* 2017). The microbiome of the rumen is given the main role in the breakdown of the complex hydrolysable components of the feed (Deryabin *et al.,* 2021), in the process of the destruction of the feed microbiome in the rumen, various metabolites (Congcong *et al.,* 2022) are produced, participating in biochemical reactions in the body and promoting the health and improvement of the productivity of the animal (Lee, 2011 and Lean and Moate, 2021). By influencing the taxonomic structure of the microbiome of the rumen, it is possible to increase the use of feed by cattle (Plottel and Blaser, 2011) and (Turnbaugh *et al.,* 2009) and, by means of next-generation sequencing (NGS), to determine the qualitative and quantitative composition of the microbiome (Bhati *et al.,* 2023; Begam *et al.,* 2022). To enhance the functional properties of the rumen, it is possible to use various substances (Bodai and Nakata, 2020) and a promising solution can also be the use of plants or plant extracts containing active components (de Vos, 2017; Atlanderova and Duskaev, 2021; Prateek *et al.,* 2022) and complexes based on them in combination with trace elements of metals (Alqaisi *et al.,* 2020 and Tuzikov *et al.,* 2021).

In the context of this, it can be emphasized that the detection, research and application of feed additives based on plants containing various phytochemical elements, in combination and separate use with trace elements of metal elements to improve the functional characteristics of the

[1]Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg-460000, Russia. [2]Institute for Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences-460000, Russia.

Corresponding Author: V.A. Ryazanov, Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg-460000, Russia.
Email: vita7456@yandex.ru

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digestive system by controlling the microbiome remains an urgent area in animal husbandry. The relevance lies in studying the effect of the feed additive *Artemisia absinthium* herbal (*A. absinthium*) (wormwood herb) and $CoCl_2$ (cobalt chloride II) on the microbiome of cattle rumen.

MATERIALS AND METHODS

The research was conducted at the Federal Scientific Centre for Biological Systems and Agrotechnology of the Russian Academy of Sciences, in the Department of Food for Agricultural Animals and Feed Technology named after Professor S.G. Leushin. The research was conducted on bulls of the breed (Kazakh white-headed) aged 13-14 months with an average living weight of 330±2.8-335±2.5 kg.

Artemisia absinthium herbal (*A. absinthium*) vegetative parts of plants, crushed to the size of 2-4 mm. and cobalt chloride (II) ($CoCl_2$) (manufacturer: NPC «Ascont+», Moscow, Russia) in the form closest to the natural, linked to amino acids and peptides, is a co-factor in enzymes that play an important role in the protective function of the animal body, growth and reproduction. *A. absinthil* (*Artemisiae absinthil* herba) - contains essential oil (up to 0.5%), it contains oxygen derivatives of bicyclic terpenes, tuyyl alcohol-tuyol, tuyol ether, tuyol esters with acetic, isovaleric, palmettic acids; from monocyclic terpenes, fellandren is present and from bicyclic sesquiterpenes - cadenene. Wormwood also contains the glycoside absintin, carotene, ascorbic acid and flavonoids. A classic bitter-spicy gastric remedy that stimulates appetite, strengthens and stimulates the activity of the digestive organs. The cobalt dosages are selected in accordance with the manufacturer's recommendations.

Animal rumen fistulas ("ANKOM Technology", d= 80 mm) were installed, with all efforts being made to reduce the damage. The experiment was carried out in four repetitions using the Latin square 4 × 4, in the amount of 4 heads. The diet for all animals consisted of 80% raw feed (seed bean 32.6%, grain feed 47.4%), concentrated feed 19.0%, 1.0% mineral additive (premium: calcium 13%, phosphorus 18.5%, sodium 12%, magnesium 3%, vitamins (× 1.000): A me 1200, D3 me 200, E mg 34, as well as B vitamins and trace elements) and the animals had free access to water. The conditions of detention and feeding standards met the requirements (Sauvant *et al.,* 2004 and Akhmetzyanova *et al.,* 2016).

The difference was that animals in trial group I were supplemented with *A. absinthium* at a dose of 2.0 g/kg of dry matter (DM), trial group II with 2.0 g/kg DM with an additional $CoCl_2$ (1.5 mg/kgDM) and trial group III with only $CoCl_2$ (1.5 mg/kgDM), write a line about the feeding of additives in different groups. The feed additive was administered during (15 day's preparatory period, 7-accounting period).

The samples of the rumen content were taken for 7 days after feeding experimental additives with the Ecohim OPA-2-20 injection syringe in 1.5 ml Eppendorf micro-tubes containing DNA/RNA Shield preservative (Zymo Research, USA). For analysis, 1.5 ml of liquid substrate from the rumen was selected for analysis, three samples for each experimental and control group.

Total DNA from rumen content samples was isolated using the Fast DNA® SPIN Kit for Faeces (MP Biomedicals Inc., USA) using the Lysing Matrix E lizing matrix. The samples were homogenized with TissueLyser LT (Qiagen, Germany). The homogenization time was increased to 5 minutes, compared to the manufacturer's protocol. The quality of the isolated DNA was tested by the method of geo-horizontal electrophoresis in 1% agarose gel and by the spectral photometric method on the device Nanodrop 8000 (Thermo Fisher Scientific, USA). The DNA concentration was measured on the Qubit 4 Fluorometer (Life Technologies, USA) using the dsDNA High Sensitivity Assay Kit.

The preparation of DNA libraries for high-performance sequencing is performed in accordance with the Illumina protocol (Part #15044223, Rev. B.). Ampicones of the V3-V4 region of the 16S rRNA gene were obtained using the primers S-D-Bact-0341-b-S-17 and S- D- Bact-0785-a-A-21 (Klindworth *et al.,* 2013). The reaction mixture (25 µl) contained 10 ng matrix; direct and reverse primers, 0.2 µM each; 80 µM DNA; 0.2 units of Q5 High-Fidelity DNA polymerase activity (New England Biolabs, USA). The DNA libraries were cleaned by solid-phase immobilization on paramagnetic particles using Agencourt AMPure XP beads (Beckman Coulter, USA). The quality of the libraries was tested by capillary electrophoresis on the Qiaxcel Advanced System (Qiagen, Germany) using the QIAxcel DNA Screening Kit cartridge. Pair-end sequencing of ampicons DNA libraries was performed on the MiSeq (Illumina, USA) platform using the Reagent Kit v.3 600-cycle (Illumine, USA) reactant set.

Bioinformatic processing of sequencing data was carried out in the following manner. Testing the quality of the source ribs using Fast QC (V. 0.11.9) [https://www.bioinformatics.babraham.ac.uk/projects/fastqc/]. Adapter cutting was carried out by cutadapt 1.9.1 (Marcel, 2011). After the removal of the adapters, the reels were re-processed by the FastQC program (V. 0.11.9), to determine the parameters of the subsequent processing. All subsequent processing steps are carried out by the USEARCH V. 11.0.667, using the UPARSE algorithm (Edgar, 2013). Left and right ridges were merged with parameters -fastq_maxdiffs 10 - fastq_pctid 80. The combined reed filtering was carried out according to the criteria: maxee 1.0 (maximum expected read error is not more than 1 in 100 nucleotides) and the minimum sicvence length is 400 p.n. Derplicated ridges have been clustered, removed chimeric sequences, operational taxonomic units (OTUs) obtained at a similarity level of 97%. To determine the representation of certain OTUs in samples, a global alignment was performed on the initial heights. The taxonomic identification of the obtained OTUs was determined using the RDP rRNA

operon database (Kerkhof *et al.,* 2022). For OTUs with low support for identification in this database, the identification was carried out in the NCBI database using the BLAST tool. From further analysis, singletons and dublons (sequences that occur once or twice) were removed.

DNA isolation, preparation of DNA libraries, equalization and bioinformatics processing of data are performed in the "Persistence of Microorganisms" CPC of the Institute of Cellular and Intracellular Symbiosis of URO RAS (Orenburg, Russia).

The sequencing results were processed using the Microsoft Excel 16 data analysis package, the Microsoft Office software (US). Numerical data were processed using the program SPSS "Statistics 20" ("IBM", USA), calculated averages (M), average square deviations (±σ), standard deviation errors (±SE). A non-parametric method of analysis was used to compare the variants. The differences were considered to be statistically significant at $p \leq 0.05$, $p<0.01$, $p<0.001$. The alpha and beta biodiversity indices were calculated using PAST 4.03 (Liu *et al.,* 2021). The regression analysis of the relationship between the proportions in the microbiome of bacteria of the predominant genus was calculated using the statistical analysis package Bio-Stat (Analystsoft) 5.9.8.5, where OTU was used as a variable.

The graphics presented in the article are based on the open source RAWGraphs 2.0 and Scimago Graphica data visualization platform (Mauri *et al.,* 2017).

RESULTS AND DISCUSSION

Taxonomic structure of rumen microbiome in control and experimental groups

A total of 161 725 sequences were obtained from 12 rumen samples and grouped into 5959 OTUs with 97% clustering. *Firmicutes, Bacteroidetes*, *Verrucomicrobia* were predominant in rumen content, which amounted to 93.1-95.9% in all samples. Filums ranging from 1 to 2% were also identified, including taxon's *Actinobacteria*, *Candidatus Saccharibacteria*, *Verrucomicrobia*, unclassified *Bacteria*, *Tenericutes, Spirochaetes, Lentisphaerae, Planctomycetes* and *Proteobacteriums*. These taxa's varied depending on the feed additives used.

In the rumen containing of control-only diets, the predominant phylum's in the microbiome were *Firmicutes*, accounting for 69.5% ($p<0.001$), *Bacteroidetes* accounted for 23.6% ($p<0.001$) and *Fibrobacteres* amounted for 2.5% of the total number of bacteria identified ($p<0.001$) (Fig 1). Additional administration of *A. absinthium* into the trial diet resulted in a 7.1% decrease in *Firmicutes* phylum in the microbiome ($p \leq 0.001$) and a 17.5% increase in *Bacteroidetes* phylum ($p<0.001$). The proportion of *Verrucomicrobia* rumen in the bacterial community has increased to 1.4% ($p \leq 0.001$) (Fig 1).

In our study, the most prominent representatives were *Firmicutes* and *Bacteroidetes*. Their ratio changed in the experimental groups when feeding *A. absinthium* and $CoCl_2$. The use of *A. absinthium* alone contributed to an increase in the proportion of bacteria at the level of genera for *Akkermansia*, *Phocaeicola*, *Alistipes*, unclassified *Bacteroidales*, unclassified *Ruminococcaceae*. Microorganisms of the rumen are given one of the main tasks in the digestion of complex carbohydrates and the consistency of the composition inhabiting the microbiome depends on the components of the diet (Wang *et al.,* 2017; Myer *et al.,* 2017; Cornejo *et al.,* 2018). So a large amount of plant fibers in the diet contributes to the growth of bacteria types *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Brandi *et al.,* 2009).

The addition of the combination of *A. absinthium* and cobalt chloride ($CoCl_2$) in the diet also resulted in changes in the level of bacterial phylum's. The proportion of microorganisms in *Firmicutes* phylum decreased significantly by 21.3% ($p \leq 0.01$). *Bacteroidetes*, on the contrary, increased sharply by 40.7% ($p \leq 0.001$). The proportion of microorganisms of the *Verrucomicrobia* phylum from the total number of bacteria identified, increased by 1.9 times. Introduction of only cobalt chloride ($CoCl_2$) as part of the main diet affected the microbiome of the rumen, with distribution by phylum's: *Firmicutes* 59.6% ($p \leq 0.05$), *Bacteroidetes* 35.9% ($p<0.01$), *Verrucomicrobia* 1.5% ($p<0.001$) (Fig 1).

The dominant genus in the rumen bacterial community in the control group were *Butyrivibrio*, *Ruminococcus*, *Saccharofermentans*, unclassified *Lachnospiraceae*, unclassified *Ruminococcaceae* and unclassified *Clostridiales*, *Mediterranea*, *Prevotella* and *Prevotellaceae* (Fig 2).

Using *A. absinthium* in the microbiome, an increase in the proportion compared to the control group was observed, for the genus *Akkermansia* 0.5% ($p \leq 0.001$), *Phocaeicola* 1.3% ($p<0.001$), *Alistipes* 0.7% ($p<0.001$), unclassified *Bacteroidales* 13.4% ($p>0.01$), unclassified *Ruminococcaceae* 17.6% ($p>0.001$) and a slight decrease in the *Prevotella* genus to 6.8% ($p=0.05$), *Fibrobacter* to 1.7% ($p=0.001$), *Butyrivibrio* to 6.1% ($p \leq 0.001$). These changes in the microbiome of the trial group resulted in changes to the structure of the dominant taxon's and an increase in the diversity of dominant families (>2%) (Fig 2).

As is well known, some species such as *Alistipes* and *Bacteroides* are resistant to bile acids (Kwa *et al.,* 2016) and some Artemisia have hepatoprotective properties, improving liver function, increasing appetite (Mulders *et al.,* 2018). In turn, the genus *Prevotella* is not able to withstand high concentrations of bile acids and their quantity decreases, which was obtained in our experience. At the same time, it is noted that the abundance of *Prevotella* leads to an increase in the formation of the intestinal hormone ghrelin, which regulates the feeling of satiety (Kholif *et al.,* 2021; Costanzo *et al.,* 2021).

The combination of *A. absinthium* and cobalt chloride ($CoCl_2$) was also included in the diet and there was an increase in the percentage of microorganisms at birth level relative to the control group for *Mediterranea* by 2 times

and *Phocaeicola* by 0.7 times. The relative abundance of *Alistipes* was 0.8 times greater than in the control group, the proportion of unclassified *Bacteroidales* increased 0.9 times and the percentage of unclassified *Ruminococcaceae* increased by 65.3% ($p \leq 0.001$) relative to the controlled group. For some births, a decrease in the microbiome ratio compared to the control group was observed. For example, the proportion of the genus *Prevotella* decreased by 95.3% ($p \leq 0.001$) and of the unclassified *Lachnospiraceae* by 74.2% ($r \leq 0.001$) (Fig 2).

The use of only cobalt chloride ($CoCl_2$) as part of the main diet affected the microbiome of the rumen. The distribution at the genus level found an increase in the proportion of microorganisms *Akkermansia*, *Alistipes*, *Phocaeicola*, unclassified *Ruminococcaceae*, unclassified *Bacteroidales* and a decrease in the number of microbial for the generation *Prevotella*, unqualified *Lachnospiriales* (Fig 2).

Indicators of alpha and beta diversity of rumen microbioms in control and experimental groups

On the evaluation of the data of predictability of different taxon's in the microbiome of rumen content of trial animals, calculated indicators of α-diversity (Table 1), characterizing the bacterial community, as well as the analysis of the basic components (PCoA) of the Bray-Curtis rumen microbiome (Fig 3).

Table 1 shows that the use of *A. Absinthium* and $CoCl_2$ leads to an increase in species diversity, the Shannon index in the trial groups was higher than the control group average by 5.8%, while the Simpson dominance index (direct) was less than 2 times in the trial groups, the selection of these feed additives did not affect the increase in the dominant species or species of rumen bacteria. Table 1 also shows how the Pielu equalization index tends to 1, which characterizes the bacterial scar community as balanced or even in number of species, which may contribute to a reduction in the burden on the digestive system.

The calculated parameters of α-diversity in our experience reflect the richness or stability of the microbiome of the rumen, which is also consistent with the results of studies in which plant additives and trace elements of metals were used, in which the ability to adapt the microorganisms of the scars is mentioned and only some species of microorganisms changes their abundance (Fei *et al.,* 2021).

To evaluate β-diversity, the main coordinate analysis was used and the species specificity of specimens of the bacterial rumen community of cattle was selected. Fig 3 clearly shows how much variation in species diversity was observed in samples using *A. absinthium* compared to the control group and how much greater influence on species diversity was shown by the addition of cobalt separately and in combination with *A. absinthium*. The location of the points of the main coordinates on the graph-fix, bacterial communities, in different planes indicates the specificity of species diversity for a separate group.

A taxonomic organization analysis in group (A) compared to the database (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/) identified the kind of microorganisms involved in lipid metabolism and butyrate production, as well as breaking down a wide range of carbohydrates.

In the group (B), with the use of *A. Absinthium*, the genus of bacteria combined with the main end products of fermentation of which were acetate, lactate, succinate, propionate, formate and hydrogen. Bacteria capable of fermenting glucose with the formation of large amounts of ethanol and milk, ant and acetic acids. There was a genus of pectinolytic bacteria. Universal microorganisms in relation to the destruction of complex carbohydrates. Glycosidase and mucin degrading bacteria.

In group (C), when combined with *A. absinthium* and cobalt chloride, a genus of degrading amino acids was combined to produce butyrate, capable of recycling different amino acids by decarboxylation or non-oxidative de-mining such as arginine, aspartate, serine, threonine with fission to L-aspartate under the action of transaminase activity, which is metabolized to fumarate and NH_3. Microorganisms that have β-glucosidase activity and form propionate. In group (D) with the use of cobalt alone, taxonomic organization is represented by genera in a similar morpho-functional state with group (C) and additionally included in this group are bacteria capable of reacting to C-glucose, D-lactose, D-sucrose, D-maltose, salicin, D-xylose, L-arabinose, esculin, glycerin, D-cellobiose, D-mannose. Having a positive reaction with α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase, N-acetyl glucosamine, amygdalin.

An increase in the unclassified *Ruminococcaceae* genus in the trial groups, which is one of the main groups of bacteria forming short-chain fatty acids capable of regulating dopaminergic neurons (Hyongjun *et al.,* 2021) may cause the opposite effect, but the study revealed a linear dependency between the mucin degrading bacteria (Colombo *et al.,* 2022); (Turnbaugh *et al.,* 2010), the *Akkermansia* genus and the unclassified *Ruminococcaceae*, which are capable of destroying mucin by releasing N-acetyl glucosamine, a component of the so-called muropeptides that are signal molecules involved in appetite regulation (Gabanyi *et al.,* 2022). Also in some works (Shabat *et al.,* 2016; Matthews *et al.,* 2019) *Akkermansia* is as an indicator of inflammatory diseases of the gastrointestinal tract, a decrease in its quantity may be accompanied by a disease, indicating its probiotic properties (Wei *et al.,* 2021).

Assessment of the relationship between the diet and the variety of microbioms of the rumen in the control and experimental groups

The indicators of the enzymatic activity of the rumen obtained in an earlier study (Ryazanov *et al.,* 2022), such as volatile fatty acids (acetic, propionic, butanoic, valerian, caproic acids), forms of nitrogen (total amount of nitrogen, non-white nitrogen, ammonia form, urea), the concentration of methane and carbon monoxide, were used to assess The effect of feed additives on the composition of the microbiome, the linear dependence of the determination index R^2 was calculated, where the values in the microbiome of the most

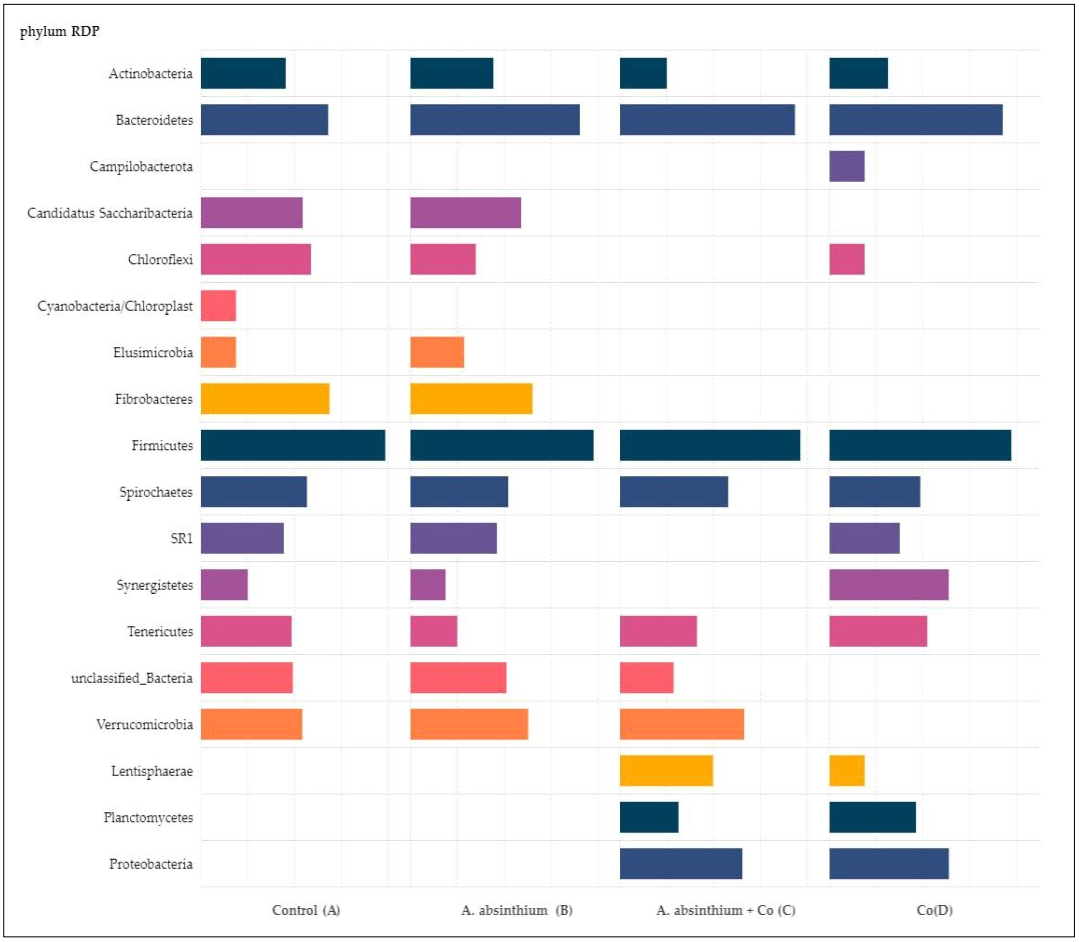


Fig 1: Relative abundance of biodiversity at the phylum level in the microbiome of cattle rumen by groups, %.

variable genera and the indicators of the enzymatic activity of the scar were analyzed. In our study, after making a linear regression between the abundance of births in the microbiome, a high correlation was established between the genus *Akkermansia* and the unclassified *Bacteroidales*, as well as between the genus unclassified *Ruminococcaceae* and the genuine *Akkermansia*, these data are consistent with previous studies of Zhang *et al.* (2019), in this same paper the authors point to the anti-inflammatory properties of the gene *Bacteroides*.

The strongest correlations between the rumen metabolites and the bacterial community were found in group (B) when using *A. absinthium* for the genera *Akkermansia* (R^2=0.37), *Phocaeicola* (R^2=0.2) and the genus *Fibrobacter* (R^2=0.2). Also in this group, the relationship of the genus unclassified *Ruminococcaceae* was found to a greater extent related to the concentrations of volatile fatty acids and amino acids (Pearson r=0.36 p= 0.0573) and for the genus unclassified *Lachnospiraceae* (Pearson r=0.43, p= 0.0165).

The combination of *A. absinthium* + $CoCl_2$ in group (C) for the genera unclassified *Clostridiales* (R^2=0.46 p=0.05, Pearson r=0.68, p=0.0001), *Akkermansia* (R^2=0.2) and *Phocaeicola* (R^2=0.24) and to a lesser extent for the genus *Mediterraneae* (R^2=0.08), in the group (D) using only cobalt for the genus *Akkermansia* (R^2=0.2), the genus *Phocaeicola* (R^2=0.12).

To establish a possible consortium between the most variable or dominant genera of rumen microorganisms, the linear regression expressed using (R^2) was calculated, among the established dependencies, the genera *Akkermansia* and unclassified *Bacteroidales* were found (R^2=0.93, the value of the t-criterion of the Student is 1.65 at p=0.0435), in group (B) when using *A. absinthium*, the genus unclassified *Ruminococcaceae* and the genus *Akkermansia* in the group when using *A. absinthium* (R^2=0.75) and in group (C) index value (R^2=0.84), Pearson correlation coefficients r=0.869 significance level p=0.05 of regression data. A decrease in the abundance of bacteria in the genus *Prevotella* revealed a regression relationship between the genus *Alistipes*, the determination index showed a value of R^2=0.3, the Phisher criterion F=5.6 with a probability of p=0.033 in the group using *A. absinthium*. A possible symbiotic relationship between honey of the genus *Prevotella* and the genus *Akkermansia* was expressed by the index of determination R^2=0.4, while the level of statistical significance of the Student's t-test was equal to 1.67 at p=0.0469 with the introduction of *A. absinthium*.

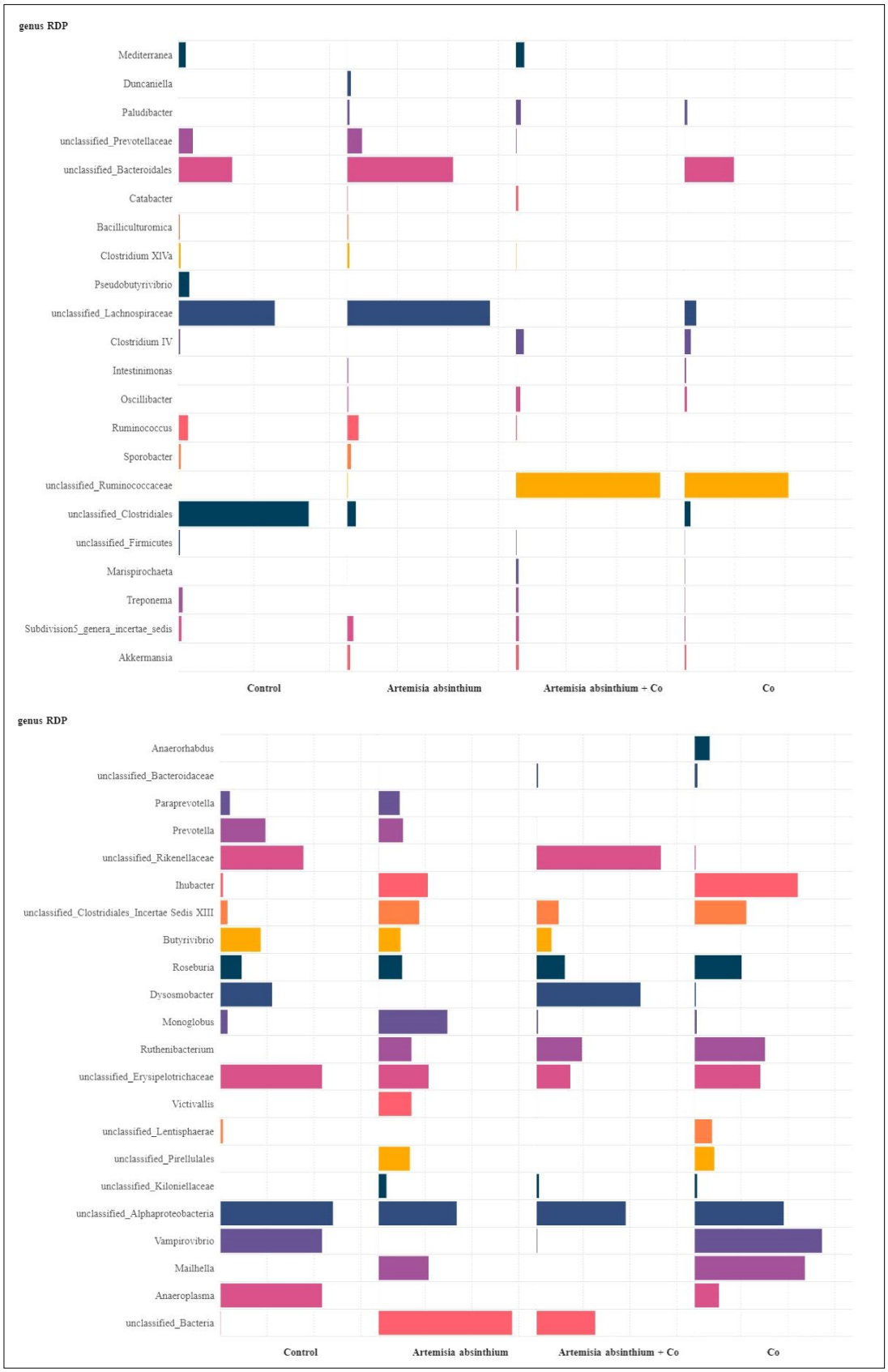
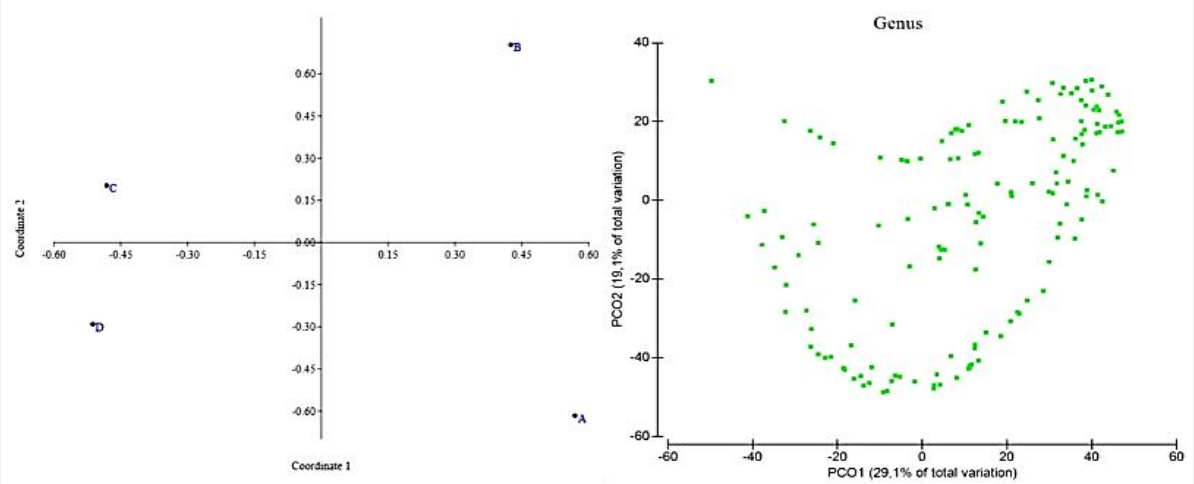


Fig 2: Relative prevalence of biodiversity at the level of the genus by group, %.



(A) control group, (B) I experimental group - *A. absinthil*; (C) II experimental group - A. *absinthil* + $CoCl_2$; (D) III experimental group - $CoCl_2$.

Fig 3: A diagram of ordering of the rumen microbiome samples, constructed using the main coordinates of the Bray-Curtis index at the birth level.

Table 1: Indices of alpha diversity of the bovine rumen microbiome.

Sample	Total species (S)	Total individuals (N)	Simpson (D)	Shannon (H)	Pielou's evenness (E)	Chao-1
A	465	17641	0.04	4.86	0.24	469
B	657	15727	0.02	5.15	0.28	663
C	408	8129	0.02	5.14	0.42	413
D	454	10762	0.02	5.15	0.39	458

Note: (A) - Control group, (B) - I experimental group- *A. absinthil*; (C) - II experimental group- *A. absinthil* + $CoCl_2$; (D) - III experimental group- $CoCl_2$.

The improved use of a feed containing a large amount of complex carbohydrates can be judged by the presence of bacteria capable of producing carbohydrate-active enzymes or CAZymes capable of destroying them (Gharechahi *et al.,* 2021; Scarpato, 2019; Thompson *et al.,* 2016). Of the large family of CAZy proteins of enzymatic activity including glycosidase and transglycosidases (Cryan *et al.,* 2019), β-glucuronides, which is a member of the family of glycosidesdase enzymes that catalyze the breakdown of the carbohydrate complex, are able to process the genus *Phocaeicola*, *Alistipes*, in our study in all the trial groups noted an increase in their number. However, the study of Conlon *et al.* (2014) and McLoughlin *et al.* (2020) suggests that an increase in the proportion of the genus *Alistipes* leads to a decrease in the concentration of butyrate and digestive system disorders with a high-protein diet. On the contrary, in the case of fermentation of a diet containing more carbohydrates this way goes differently (Petrič *et al.,* 2021), the resulting products of the breakdown of carbohydrates support homeostasis, in our study the diet contained more than rough feed. Digestive disorders are more associated with the fermentation pathways and the presence of other genes of microorganisms than the genus *Alistipes* itself (Kang *et al.,* 2016). And the decrease in the proportion of the genus *Alistipes* can indirectly affect the peristaltic of the intestine (Schneeberger *et al.,* 2015; Parker *et al.,* 2020) deteriorating its function.

CONCLUSION

The results of the study show that the use of *Artemisiae absinthium* plant as a feed additive both separately and in combination with cobalt changes the taxonomic structure of the cattle rumen bacteria. This is due to changes in the diversity of microbial species of individual functional genera of bacteria involved in the breakdown of carbohydrates, which are able to improve enzymatic characteristics. A symbiotic relationship has also been established for separate genera of rumen microorganisms involved in the digestive process.

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Approval of the ethics committee

The study was conducted with the permission of the ethics committee of Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Protocol No. 2 dated 10.11.2022.

Conflicts of interest

Authors declares that they have no conflict of interest.

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