THE EFFECT OF A MIXTURE OF ESSENTIAL OILS OF *CORIANDER* AND *FENNEL* ON THE MICROBIOME OF CALVES.

Ostrenko K.S.[1*], Ovcharova A.N.[1], Kutin I.V.[1], Koltsov K.S.[1], Belova N.V.[1] Skipor O.B.[2], Nevkrytaya N.V.[2]

Abstract.
The aim of the study was to assess the effect of phytogens of essential oil crops – fennel (Foenículum vulgáre) and coriander seed (Coriándrum sátivum) on the formation of microbiota in calves in the dairy period. The studies were carried out on calves of the dairy period of the black-and-white breed, on the basis of the laboratory of immunobiotechnology and microbiology of VNIIFBIP animals in 2023. The determination of the microbiome of the scar content was carried out by T-RFLP analysis. In the course of the conducted studies, there was a tendency to increase the live weight of calves of the experimental groups under the influence of the fed factor: the maximum increase over the entire study period was in animals of the 3rd experimental group, which was 2.7% higher compared to analogues from the control group. The results of the T-RFLP analysis showed that an increase in cellulolytic bacteria is already observed in the rumen of 2-month-old calves. In the experimental group, their quantities are almost 2 times greater ($P \leq 0.05$) compared to calves in the control group. Based on the data obtained, it can be concluded that the use of feed complexes from essential oils of coriander and fennel usually has a positive effect on the formation of the microbiome of the calf rumen.

Keywords: calves, microbiome, essential oils, coriander, fennel.

[1*]All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Animals — Branch of the L.K. Ernst Federal Research Center for Animal Husbandry, VNIIFBiP, Borovsk 249013, Russia
[2]FSBSI "Research Institute of Agriculture of Crimea"; 150, Kievskaya str., Simferopol, Republic of Crimea, 295453, Russia;

***Corresponding Author:** Ostrenko K.S.
*All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Animals — Branch of the L.K. Ernst Federal Research Center for Animal Husbandry, VNIIFBiP, Borovsk 249013, Russia
E-mail:ostrenkoks@gmail.com, 89109166658.

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Antibiotics have been traditionallyused as a method of combating pathogens, it has been shown that they contribute to the development of rumen, improve the productivity of calves and reduce the risks of diarrhea of alimentary etiology, which makes it possible to increase the safety and productivity of calves. Concerns about the development of antibiotic resistance of pathogenic flora have prompted the World Health Organization (WHO) to limit the use of antibiotics for veterinary purposes and completely abandon their use as growth stimulants in farm animals. Thus, there is an urgent need to search for substances that have an antibiotic-like effect, but do not affect the antibiotic resistance of microorganisms. One of the possible solutions to this problem may be the use of essential oils (EO) in various combinations of essential oils (CEO) [1,2].

Essential oils have become an active area of research due to their ability to alter the metabolism and growth of bacteria. One such example is the inhibition of the growth of Escherichia coli, one of the main representatives of the animals' gastrointestinal microflora, many strains of which have pathogenic properties. In addition, there is evidence that the oregano preparation can be as effective as neomycin in the prevention of infectious diseases and that EO limit the possibility of developing resistant strains of bacterial populations, which makes them an ideal candidate for further studying [3,4].

The international literature presents data on the effect of essential oils to stimulate appetite, intensify the consumption of starter feed in calves, increase feeding efficiency and increase body weight gain, as well as an increase in the number of beneficial bacteria in the intestinal flora [5,6].

In solving this problem, it is important to choose essential oils taking into account their effectiveness, cost and production volumes (availability for purchase).

Essential oil plants of the Celery family are widespread. Thus, coriander *Coriandrumsativum L*. occupies in Russia up to 90% of all areas occupied by essential oil-bearing crops [7]. The main component of its essential oil is linalool (about 70%). It's known that linalool and linalool-rich essential oils exhibit antimicrobial, anti-inflammatory, anti-cancer, and antioxidant properties [8]. The results of studies with evidence of a positive effect on the central nervous system provide the most promising potential for the treatment of diseases. The effectiveness of linalool has been established in model animals; its antihyperalgesic and antinociceptive effects have been shown [9]. Thus, linalool has great potential for its use as a natural and safe alternative to therapeutic agents [10].

A related crop to coriander is common fennel *Foeniculumvulgare Mill*. The main component (about 70%) contained in its essential oil is anethol. This substance has powerful anti-inflammatory and neuroprotective properties, has an antinociceptive effect on neuropathic pain. A study on mice showed that anethol in the treatment of chronic constriction injury eliminates the sciatic nerve damage, improves nerve's conduction [11]. It has been established that trans-anethol has the ability to disrupt bacterial communication and can be recommended as a new component to combat *P. Aegidinosa* and other clinically significant pathogens [12].

The information available in the literature data allowed us to start a study on the use of coriander and fennel essential oils as additives to feed young cattle. The first stage of a comprehensive study showed a positive effect of these biologically active substances. When they were introduced into the feed at a dose of 1 ml per head per day, an increase in weight gain was noted, as well as an increase in the immune response [13-14].

The aim of the research was to develop a complex phytogenic feed additive based on phytogens of essential oil-bearing crops – common fennel (Foenículumvulgáre) and coriander (Coriándrumsátivum) (using varieties of breeding "FSBI "Scientific Research Institute of Agriculture of the Crimea" - NIISH of the Crimea) and to study their effect on the formation of microbiota in calves in the milk period.

Materials and methods of research.

The research was carried out on calves of the milk period of the Russian Black Pied breed based on the laboratory of immunobiotechnology and microbiology and the vivarium of VNIIFBIP of animals in 2023.

4 groups of dairy calves of pairs of analogues were formed, 10 heads each.

From the age of 3 weeks, the calves of the experimental groups were injected with a calf milk replacer (CMR) of 10 ml per head of a feed complex based on essential oils of common fennel *(Foenículumvulgáre)* and coriander *(Coriandrumsativum)* (using varieties of breeding

"FGBUN "Scientific Research Institute of Agriculture of the Crimea" - NIISH of the Crimea). The water-soluble emulsion of the feed complex contained 1 ml of an essential oils mixture. The scheme of the experience is presented in Table 1.
The main diet of calves consisted of CMR (750 g of dry CMR per head per day), 150 g of starter compound feed per head per day as complementary food and alfalfa hay.

The duration of the study was 30 days. Weighing of calves was carried out before the start of the study and at the end of the 1st stage of the study. Sampling of the rumen content was carried out at the end of this stage of the study. Samples of the rumen content were taken using a rumen probe.

DNA isolation from the rumen content was carried out in the laboratory of immunobiotechnology and microbiology. The total DNA for T-RFLP analysis was isolated using the "Genomic DNA Purification Kit" ("Fermentas", Lithuania).

The determination of the rumen content microbiome using the T-RFLP method was done with the participation of employees and on the equipment of "Biotrof +" LLC.

Table 1 – Researchscheme

Group	Headsinthegroup	Feedingcharacteristics
Scientific experience on veal-calves – duration of 30 days		
1 - experimental	10	BD +10 ml emulsion of fennel and coriander oils in liposomes in a ratio of 70 to 30 %
2 – experimental	10	BD +10 ml emulsion of fennel and coriander oils in liposomes in a ratio of 50 to 50 %
3 – experimental	10	BD +10 ml emulsion of fennel and coriander oils in liposomes in a ratio of 30 to 70 %
4 - control	10	Basicdiet (BD) per head

DNA amplification for T-RFLP analysis was performed using the Verity device ("Life Technologies, Inc.", USA). T-RFLP analysis was performed using the Beckman Coulter CEQ-8000 Analyzer (USA).

Statistical processing. The obtained digital data were processed by the method of variational statistics. To identify statistically significant differences, the Student's criterion according to N.A. Plokhinskywas used. We used the office software package "Microsoft Office" with the program "Excel" ("Microsoft", USA). The results were considered reliable, starting with a value of $p < 0.05$.

Research results.
In the course of the conducted studies, it was found that the use of various combinations of coriander and fennel essential oils when introduced into the CMR at the first introduction reduced the volume of the diluted product consumed by calves. This decrease is due to the sharp smell of a mixture of essential oils. In this connection, it is necessary to accustom animals to the consumption of this product and introduce it into the diet, gradually increasing the dosage. We dosed the mixtures for 5 days until the required concentration was reached. After getting used to and forming a stable feeding reflex, the rate and volume of consumption in the experimental groups did not differ from the indicators in the control group.

The use of the studied complex emulsions of essential oils affected the productivity indicators in calves of the milk period. The data is presentedin Table 2.

Table. 2 Weight indicators of calves in the milk fattening period (M ± m, n = 10).

Groups	1 experimental	2 experimental	3 experimental	Control group
Startingweight (kg)	56,5±1,9	56,3±1,6	55,8±2,6	56,5±1,9
Average weight after 30 days (kg)	71,9±1,1	72,3±1,7	72,8±2,4	70,9±1,6
Gain over the period (kg)	15,4±0,8	16,5±1,2	17,0±1,7	14,4±0,8
Averagedailygain (g)	0,51±0,02	0,55±0,04[a]	0,57±0,05[a]	0,48±0,02
% by gain to control	101,4	102,0	102,7	100

Means (±standard error) within a row (overall) followed by different superscript are significantly different,
general linear model (GLM), $p < 0.05$, Significant to Group: a - Control group, at $p < 0.05$, according to Tukey's test.

The use of essential oils in various combinations showed differences in weight indicators and average daily gains over the entire study period. Based on the results presented in Table 2, it can be concluded that the data on live weight before the experiment in animals of all groups did not differ significantly. At the end of the first stage of the study, there was a tendency to increase the live weight of calves of the experimental groups under the influence of the fed factor: the maximum increase was in animals of the 3rd experimental group, which was 2.7% higher compared to analogues from the control group. In the first and second experimental groups, positive dynamics was also observed in terms of gains of 1.4 and 2% relative to the indicators in the control group. Average daily gains in the 2nd and 3rd experimental groups were significantly higher by 14.6 and 18.6% ($p < 0.05$), relative to the data in the control group. An increase in the average daily gains indicates an increase in the efficiency of the CMR digestibility and activation of the calves' digestive system. The data obtained confirm that the animals of the experimental groups used the nutrients of the feed better for the increase in live body weight.

The use of feed compositions of essential oils also had a beneficial effect on the formation of the rumen microbiota in calves, which is confirmed by the data of T-RFLP analysis. The results of T-RFLP analysis of the calves' bacterial community of the experimental and control groups are presented in Table 3.

Table 3. Total microbiota content in the rumen of calves of experimental and control groups, % (M ± m, n=10)

	1st group	**2nd group**	**3rd group**	**Control**
Selenomonads, Veillonella	9,90 ± 2,36	9,98 ± 2,16	10,30 ± 2,18	9,88 ± 1,44
Cellulolytics	30,08 ± 3,05 [a]	32,08 ± 3,12 [a]	35,08 ± 3,00 [a]	18,77 ± 1,54
Bifidobacteria	0,94 ± 0,45 [a]	1,44 ± 0,65 [a]	1,64 ± 0,35 [a]	0,69 ± 0,22
Bacilli	21,85 ± 6,00	22,45 ± 5,24	22,88 ± 4,15	20,41 ± 2,44
Actinobacteria	5,74 ± 2,23 [a]	5,74 ± 2,13 [a]	4,74 ± 2,13 [a]	10,20 ± 1,47
Enterobacteria	4,26 ± 0,93 [a]	4,26 ± 0,83 [a]	3,11 ± 0,87 [a]	5,67 ± 0,81
Lactobacilli	2,29 ± 0,84 [a]	2,59 ± 0,74 [a]	2,99 ± 0,91 [a]	5,01 ± 2,22
Mycoplasma	2,10 ± 0,75	1,7 ± 0,85	1,6 ± 0,77	2,93 ± 0,25
Staphylococci	1,01 ± 0,47	0,71 ± 0,52	0,91 ± 0,45	1,00 ± 0,20
Fusobacteria	2,42 ± 0,78[a]	1,42 ± 0,73 [a]	1,28 ± 0,62 [a]	2,41 ± 0,69
Peptococcaceae	0,79 ± 0,37	0,74 ± 0,29	0,69 ± 0,35	0,41 ± 0,19
Campylobacteria	0,53 ± 0,20 [a]	0,53 ± 0,25 [a]	0,73 ± 0,22 [a]	0,20 ± 0,13
Pseudomonas	2,48 ± 0,45 [a]	1,48 ± 0,46 [a]	1,18 ± 0,41	1,25 ± 0,40
Pasterella	1,46 ± 0,61 [a]	1,43 ± 0,67 [a]	1,44 ± 0,57 [a]	3,19 ± 0,93
Uncultivatedbacteria	14,75 ± 3,33	12,75 ± 3,17	10,95 ± 3,13	13,10±3,45

Means (±standard error) within a row (overall) followed by different superscript are significantly different, general linear model (GLM), $p < 0.05$, Significant to Group: a - Control group, at $p < 0.05$, according to Tukey's test.
Cellulolytic bacteria are the dominant bacteria of the rumen of ruminants that break down the fiber of plant feeds to volatile fatty acids (VFA). The following bacterial families were mainly represented in the rumen contents of the studied calves: *Clostridiaceae, Prevotellaceae, Eubacteriaceae, Ruminococcaceae, Lachnospiraceae*, as well as representatives of the *Bacteroidetes*phylum.

One of the signs describing the microbiocenosis of the "adult type" in ruminants is its ability to break down cellulose.

The total content of cellulolytic bacteria in the animals of the control group was at the lower level of the norm for the corresponding age, and in the calves of the experimental groups - almost one and a half to two times higher.

The results of our analysis showed that in the rumen of two-month-old calves, there is already a significant number of bacteria that are involved in the fermentation of cellulose to volatile fatty acids; in the experimental groups, their amounts are almost 2 times greater (P≤0.05) compared to the calves in the control group.

There is also an increase in the normoflora compared to the calves of the control group, so the number of bifidobacteria in the experimental groups was 36.2% higher and further the increase was several times higher (P≤0.05) compared with the indicators in the control group.

The results obtained by the molecular genetic method in this study coincide with the data obtained earlier by a number of researchers [16].

Bifidobacteria and bacilli in the digestive tract exhibit the following properties: antimicrobial, immunomodulatory activity, synthesis of vitamins, synthesis of some indispensable amino acids, protect the body from threats from pathogenic and opportunistic microflora. The largest number of representatives of the normoflora was significantly higher in calves of the 3rd experimental group where a mixture of coriander and fennel essential oils was used in a ratio of 70 * 30.

Conclusion.

Based on the data obtained, it can be concluded that the use of feed complexes of coriander seed and fennelessential oils usually has a positive effect on the rumen microbiome similar to the use of probiotic drugs. The use of CEO increases the level of protection of the rumen microbiome and the body of dairy calves from negative stress factors, which affects productivity and sets a positively directed vector in fattening calves.

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