

MICROBIOTA OF SHEEP RUMEN IN CONDITIONS OF KEEPING IN A PEN AND ITS CORRECTION BY THE INTRODUCTION OF THE ADAPTOGEN LITHIUM ASCORBATE INTO THE RATION

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Lithium ascorbate is a stress-protective adaptogen with neurotrophic, neuroprotective, antioxidant, and immunomodulatory properties.

The existence of the "microbiota-gut-brain" axis has been proven. It is assumed that the adaptogen lithium ascorbate can affect all parts of the "microbiota-gut-brain" axis, which will simultaneously solve problems associated with disorders that occur when exposed to stress of various etiologies, and prevent gastrointestinal disorders caused by changes in the microflora.

The feed additive will increase the profitability of production in sheep farming and approach the solution of problems of increasing rumen digestion.

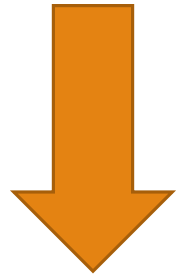
Goals and objectives of the research.

The aim of the study was to obtain new knowledge about the composition of the microbiota of sheep rumen, to find out the **effect of the adaptogen lithium ascorbate on the qualitative and quantitative composition of the microflora of sheep rumen** by molecular genetic methods.

The scheme of the experiment

Group	animals in the group	Feeding characteristics
1 - control	8	The main diet (MD)
2 - testing	8	MD +10 mg LiAsc per kg of live weight

The duration of the experiment is 60 days
Isolation of DNA from the rumen content



NGS



Main ration:concentrate-500 g, mixed grass hay-1.5 kg

Composition of granulated compound feed
"Compound feed for goats and sheep KK-85 (MRS)"

Composition of the recipe		Additionally, vitamins and trace elements were added to 1 kg of mixed feed	
Denomination	%	Denomination, measure unit	Quantity
Oats	15,0	Fe, mg	25,0
Barley	46,0	Cu, mg	2,5
Sunflower oil	1,0	Zn, mg	30,0
Wheat bran	16,0	Mn, mg	40,0
Sunflower meal, СП36%, СК 19	15,5	Co, mg	1,0
Herbal Flour	5,5	I, mg	1,0
Defluorinated phosphate	2,0	Se, mg	0,15
Limestone flour	3,0	Vitamin A, тыс.МЕ	10,0
Premix	0,5	Vitamin D3, тыс.МЕ	1,0
Table salt	1,0	Vitamin E, mg	10,0
		S, mg	100,0

**Weight indicators of sheep against the background of the use
of lithium ascorbate, kg**

Groups	The weight of the animals		
	before the experiment	After 30 days	After 60 days
Control	37,11±1,09	38,35 ± 2,33	38,59 ± 2,57
LiAsc	37,59 ± 1,7	38,94 ± 2,92	39,83 ± 3,81

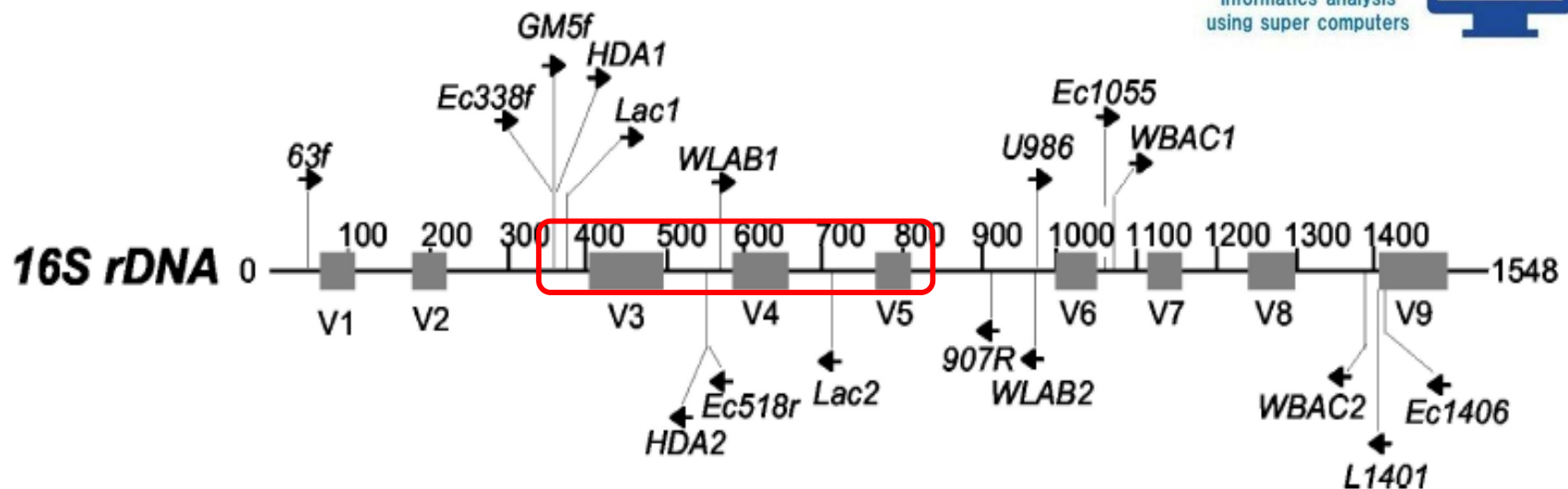
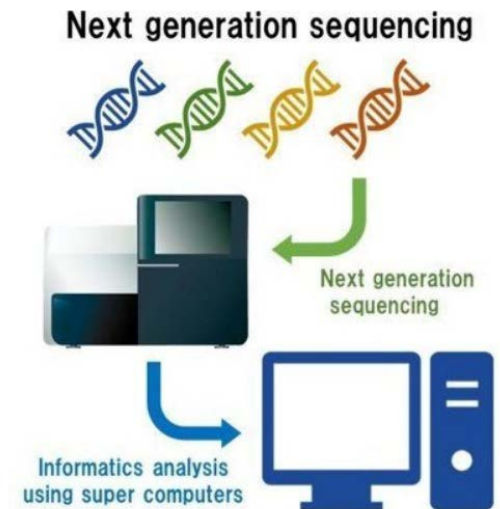
NGS-sequencing

The aim of the study was to analyze the structure of the bacterial community in the rumen contents of sheep using the molecular genetic method of NGS-sequencing.

Target: 16S rRNA gene

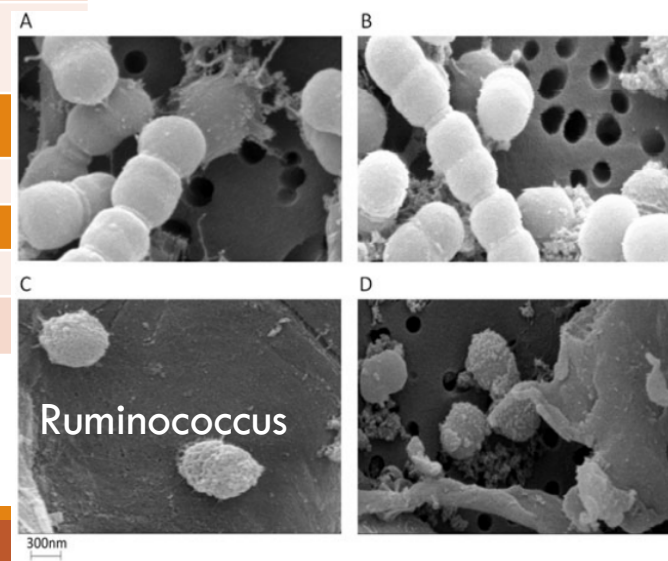
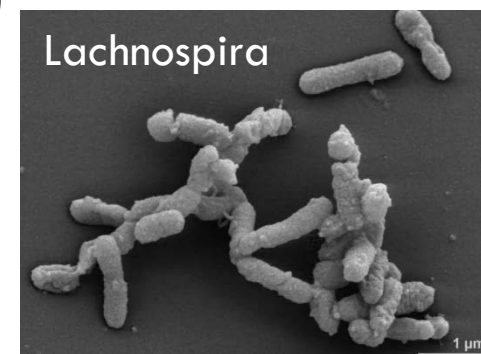
Direct primer: **343F** (5'-CTCCTACGGRRSGCAGCAG-3')

Reverse primer: **806R** (5'-GGACTACNVGGGTWTCTAAT-3'),
(amplified from 343 to 806 nucleotide-about 460 bp, V3V5)

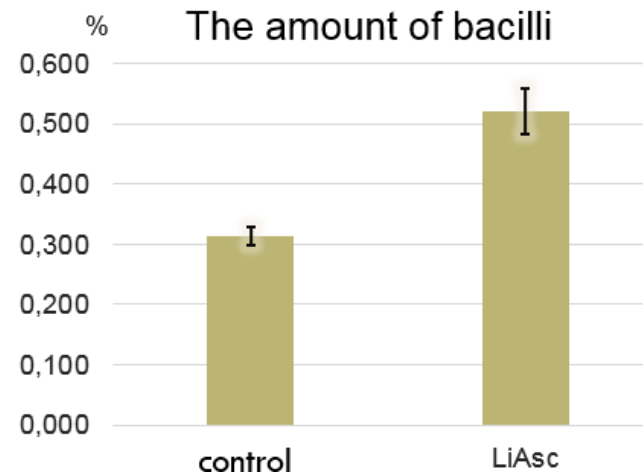
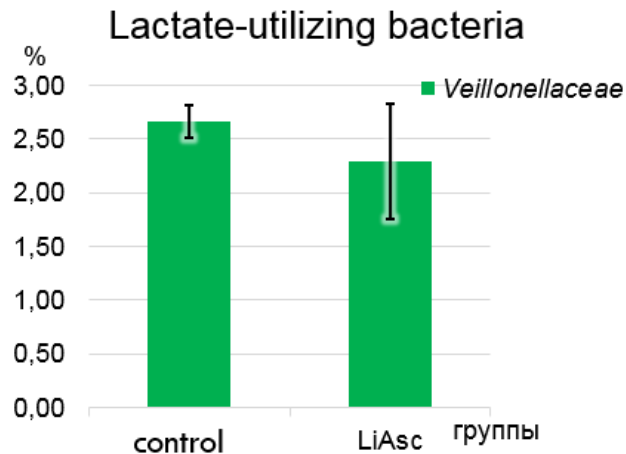


Normoflora of sheep rumen of the control and experimental groups (% of the total number of bacteria)

	Control		LiAsc	
Group	M	± m	M	± m
Cellulose-lysing bacteria				
<i>Bacteroidetes</i>	55,63	± 2,02	57,69	± 1,89
<i>Ruminococcaceae</i>	11,96	± 0,72	11,89	± 1,25
<i>Lachnospiraceae</i>	4,37	± 0,27	3,86	± 0,19
<i>Clostridiaceae</i>	1,22	± 0,06	0,95	± 0,18
<i>Eubacteriaceae</i>	0,42	± 0,02	0,98	± 0,16
<i>Peptostreptococcaceae</i>	0,02	± 0,00	0,04	± 0,02
<i>Thermoanaerobacterales</i>	0,02	± 0,01	0,01	± 0,00
The amount of cellulolytic	73,64	± 1,62	75,42	± 0,94
Lactate-utilizing bacteria				
<i>Veillonellaceae</i>	2,67	± 0,15	2,29	± 0,53
Other				
<i>Bifidobacteriales</i>	0,002	± 0,002	0,005	± 0,007
The amount of bacilli	0,31	± 0,02	0,52	± 0,04



Normoflora of the rumen of sheep



Content of "undesirable" and conditionally pathogenic microflora, %.

	Control		LiAsc	
	M	± m	M	± m
Lactobacillales	0,570	± 0,097	0,423	± 0,255
Enterobacteriaceae	0,044	± 0,016	0,019	± 0,003
Actinomycetales	0,076	± 0,011	0,079	± 0,032

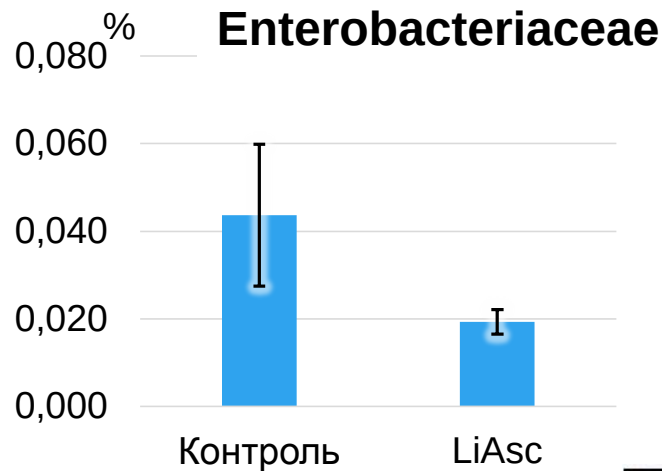
Lactobacilli in the rumen of small cattle ferment monosaccharides to lactic acid and can lead to a decrease in the pH in the rumen, so they are undesirable.



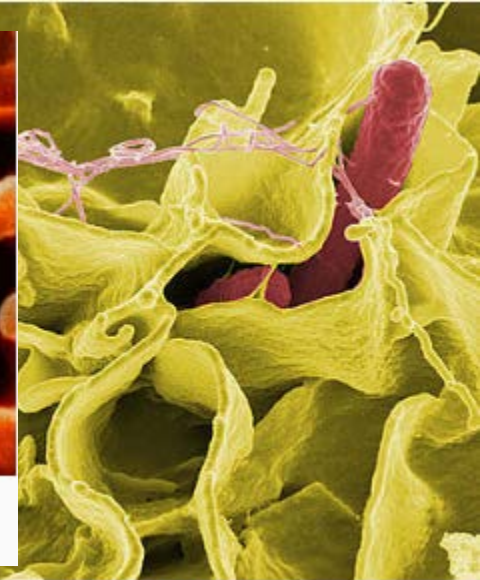
Content of "undesirable" and conditionally pathogenic microflora, %.

Enterobacteria is a family of Gram-negative rod-shaped spore-forming bacteria, facultative anaerobes. In addition to the normal microflora, it includes a significant number of pathogenic microbes: shigella, Salmonella and some types of Escherichia.

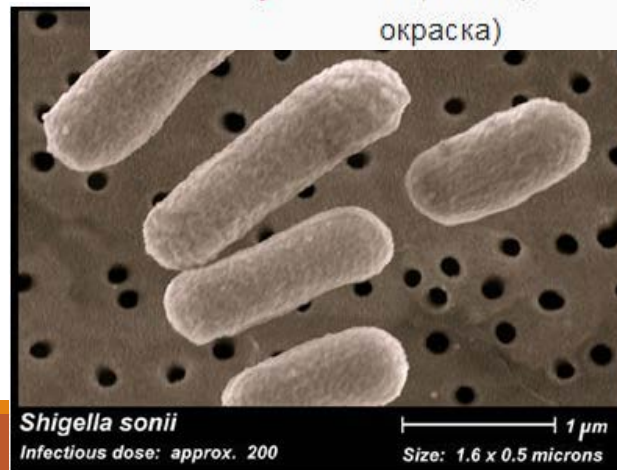
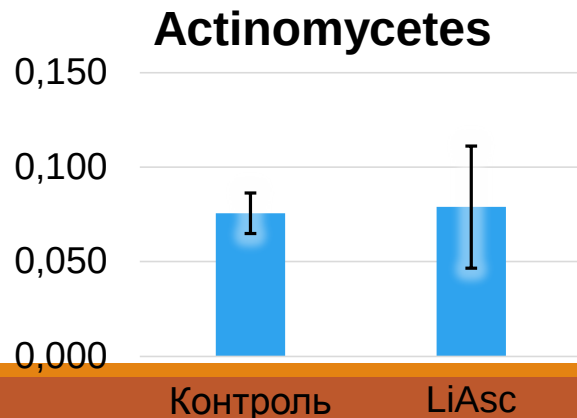
Actinomycetes (*Actinomycetales*) — ferment carbohydrates to form an acid without gas, the fermentation products are acetic, lactic, formic and succinic acids.



Actinomyces israelii, СЭМ (компьютерная окраска)

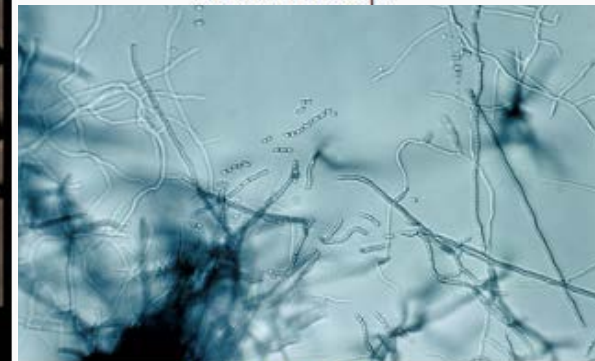


Salmonella sp.



Shigella sonii
Infectious dose: approx. 200

Size: 1.6 x 0.5 microns

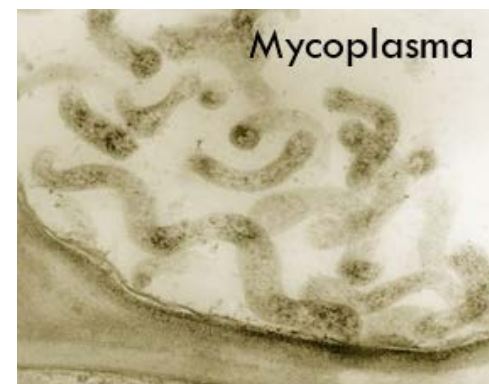


Streptomyces sp.

Pathogenic microorganisms

The content of groups of pathogenic microorganisms in the rumen fluid of sheep of the control and experimental groups, %.

Group of pathogens	Pathogens	Control		LiAsc	
		M	± m	M	± m
Mycoplasma	Anaeroplasma bactoclasticum	0,81	± 0,03	0,16	± 0,03
	Anaeroplasma sp.	0,12	± 0,01	0,11	± 0,04
	An. abactoclasticum	0,08	± 0,01	0,03	± 0,01
	Mycoplasma sp.	0,03	± 0,00	0,03	± 0,00
	Mycoplasma penetrans	0,02	± 0,00	0,01	± 0,01
Moraxelles	Acinetobacter sp.	0,02	± 0,02	0,04	± 0,02
Pseudomonads	Pseudomonas sp.	0,03	± 0,00	0,04	± 0,01
Staphylococcus	Staphylococcus sp.	0,01	± 0,01	0,02	± 0,01
Streptococcus	Strept. acidominimus	0,06	± 0,01	0,06	± 0,05
	Streptococcus sp.	0,05	± 0,01	0,03	± 0,01
Succinivibrio	Succinivibrio dextrinosolvens	0,05	± 0,03	0,03	± 0,01
Treponema	Treponema sp.	0,60	± 0,02	0,33	± 0,05
	Treponema bryantii	0,09	± 0,03	0,12	± 0,01
	Treponema parvum	0,14	± 0,04	0,06	± 0,03
	Trep. isoptericolens	0,16	± 0,12	0,03	± 0,02
	Treponema porcinum	0,05	± 0,01	0,06	± 0,01
	Trep. amylovorum	0,03	± 0,01	0,05	± 0,03
	Trep. saccharophilum	0,05	± 0,03	0,03	± 0,01
	Trep. maltophilum	0,02	± 0,01	0,01	± 0,01
Holdemania	Holdemania sp.	0,02	± 0,00	0,03	± 0,01
Erysipelotrix	Kandleria vitulina	0,03	± 0,02	0,06	± 0,02
	Solobacterium sp.	0,02	± 0,01	0,07	± 0,04
Other		0,19	± 0,04	0,17	± 0,02
Total		2,65	± 0,46	1,58	± 0,45



As a result of NGS sequencing in the rumen of sheep were found:

The proportion of bacteria and archaea in the rumen content of sheep of the control and experimental groups (% of the total number of microorganisms)

Group	Control		LiAsc	
	M	m	M	m
Bacteria	95,40	0,15	95,89	0,53
Archaea	0,18	0,02	0,24	0,06

37 **phylums** of bacteria, including 20 phylums with a share above 0.1% of the total number of microorganisms; the main phylums are Bacteroidetes (53-60%) and Firmicutes (24-29%)

- 76 **classes** of bacteria (24 classes with a fraction higher than 0.1%);
- 98 **orders** of bacteria (28 orders with a fraction above 0.1%);
- 228 **families**;
- 660 **genus**;
- 894 **species**.

Conclusion

- ❖ Groups of microorganisms that promote the digestion and assimilation of feed (cellulosolytic bacteria, lactate-fermenting bacteria, beneficial bacilli) were present in the rumen content of sheep of the control and experimental groups in uniform and high amounts. Thus, useful cellulosolytics were detected by at least 70% of all detected microorganisms, lactate-fermenting bacteria-at least 1.7%, useful bacilli - at least 0.3%.
- ❖ It was found that the introduction of lithium ascorbate into the main diet of sheep contributed to a decrease in the content of pathogenic and some conditionally pathogenic bacteria.
- ❖ The results obtained give grounds to conclude that lithium ascorbate has a positive effect on the qualitative and quantitative composition of the sheep rumen microbiota, and to recommend its use in addition to the main diet of sheep both under satisfactory conditions of maintenance and under stress.



Thanks for your attention!