«АГРАРНАЯ НАУКА – СЕЛЬСКОМУ ХОЗЯЙСТВУ»
XVII МЕЖДУНАРОДНАЯ НАУЧНО-ПРАКТИЧЕСКАЯ КОНФЕРЕНЦИЯ

THE EFFECT OF FEED ADDITIVE ON THE NORMALIZATION OF THE MICROBIOTA OF THE RUMEN OF DAIRY CALVES

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Increasing the efficiency of meat and dairy farming is closely related to the improvement of animal feeding technologies.

Optimization of protein nutrition and metabolic processes in the body of ruminants by creating conditions for the effective use of nitrogenous feed components, reducing the release of unused nitrogen with excrement is an urgent task of agricultural science.

The protein of the feed consists of proteins and amides. Protein is an essential source of amino acids for protein synthesis of animal origin. Amides are a non-protein part of protein that is important for ruminants.

Protein metabolism is an integral part of nitrogen metabolism, the end products of which are **urea** and **ammonia**.

Ammonia is a toxic compound. Its maximum permissible blood level is 60 μmol/l.

The level of urea cycle intermediates, including toxic ammonia, can be regulated by affecting the activity of key enzymes of the cycle.

N-carbamoyl glutamate (NKG) (a non-metabolizable analog of N-acetylglutamate, an allosteric activator of the first enzymatic reaction of the urea cycle) is an effective agonist and regulator of carbamoyl phosphate synthetase-1.

This is especially important for ruminants:

- the microbiota of the rumen releases ammonia as a result of fermentation of feed proteins
- the microbiota of the rumen is able to utilize urea.

NKG activates the urea cycle: its use as a feed additive ensures a more complete conversion of urea nitrogen and ammonia into endogenous protein and increases the meat productivity of farm animals.

NKG significantly improves the growth and development of calves, however, its effect on the microbiota of the rumen and other parts of the gastrointestinal tract has not been studied.

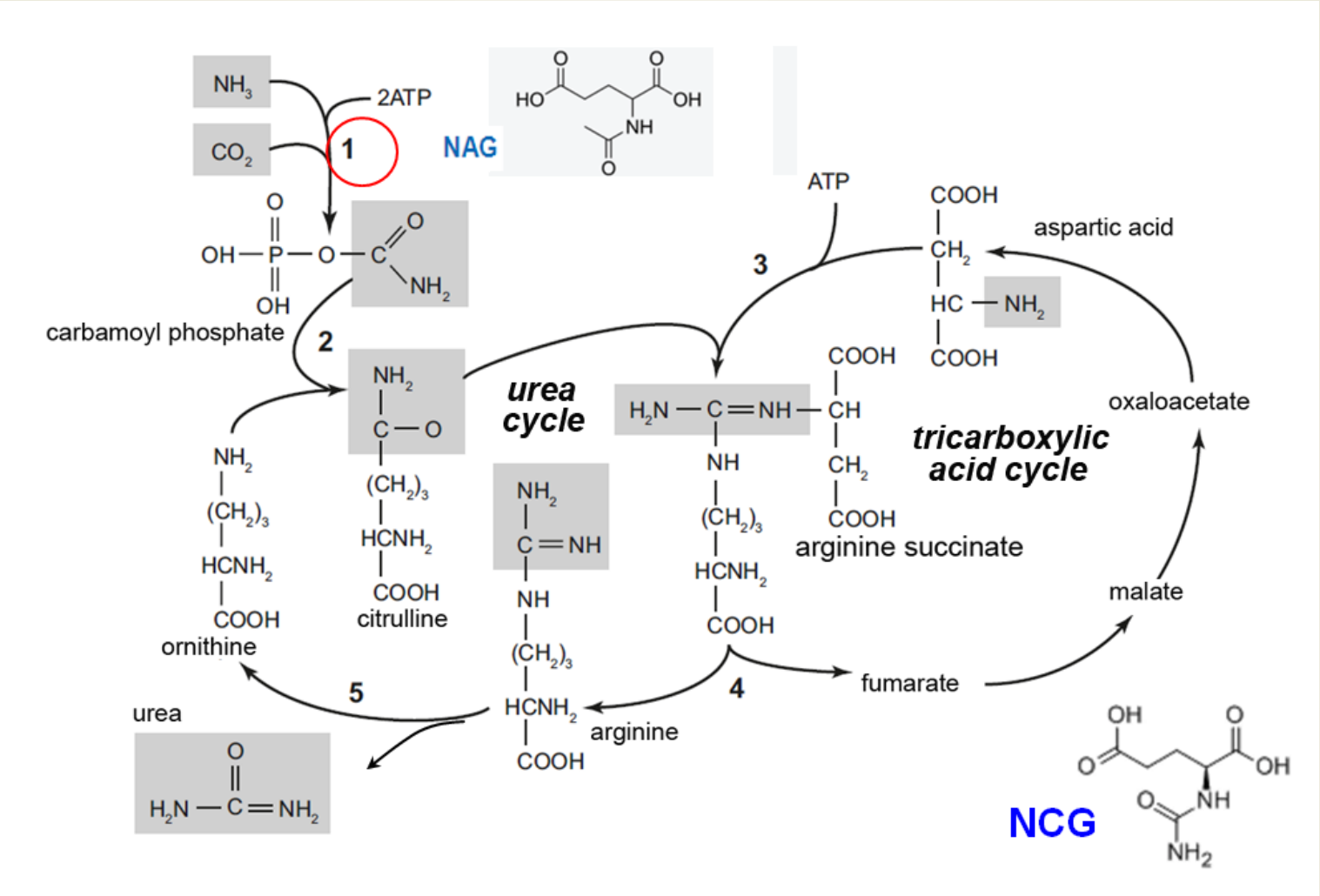


Fig.1. **The urea cycle and its relationship with the tricarboxylic acid cycle.** It occurs in liver cells: the first two reactions are in the mitochondria, the rest are in the cytosol. It begins with the formation of an energy-rich carbamoyl phosphate in the presence of N-acetylglutamic acid (NAG, cofactor), with the participation of the enzyme **carbamoyl phosphate synthetase 1** (1). Carbamoyl phosphate in reaction with ornithine forms citrulline (2 - ornithine transcarbamoylase). Citrulline and aspartic acid (from TAC) form argininosuccinate (3 - arginine succinate synthetase), which is cleaved into fumaric acid (in TAC) and arginine (4 — arginine succinate lyase). Arginase (5) splits arginine into urea and ornithine, which can re-enter the mitochondria and start a new urea cycle

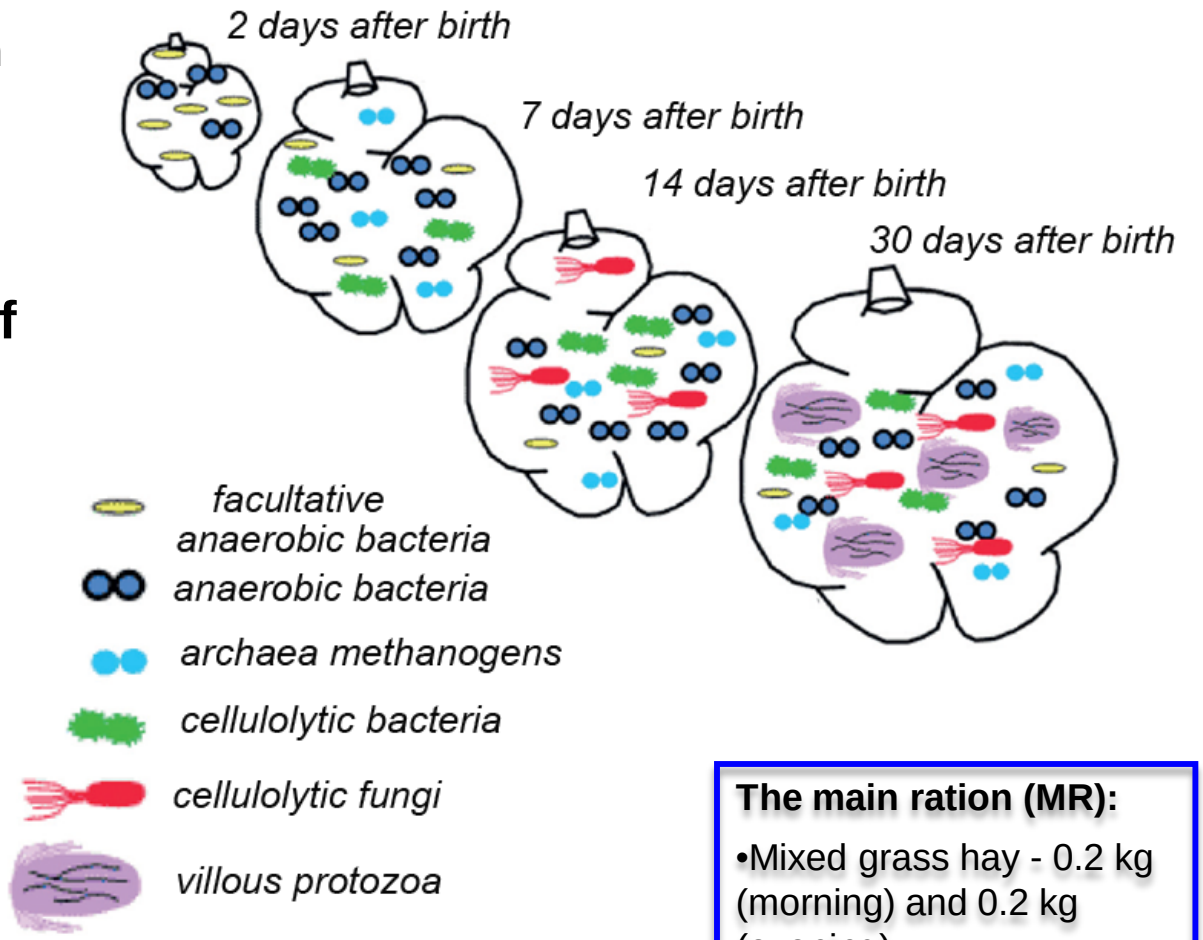
The rumen is the main digestive organ of ruminants.

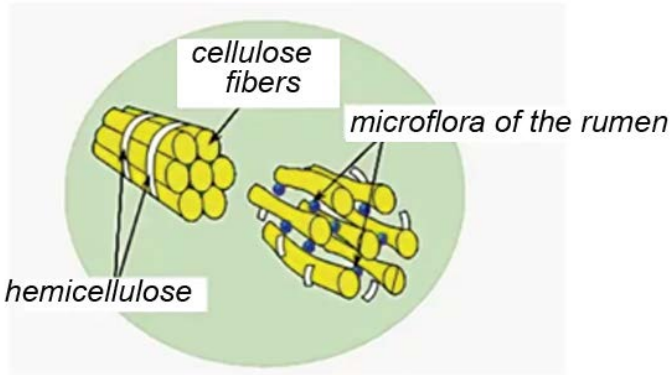
Colonization by microorganisms of calve rumen

The microbiota of the rumen changes in parallel with changes in feeding, housing conditions, with the age of animals, under stress.

The rumen environment can change the composition and microbial activity with its physico-chemical parameters.

All ruminants have an individual microbial balance of the rumen





The main ration (MR):

- Mixed grass hay - 0.2 kg (morning) and 0.2 kg (evening).
- Compound feed - 1.0 kg (morning) and 1.0 kg (evening).
- Whole milk substitute – 3.0 liters (morning) and 3.0 liters (evening).
- Water is freely available.

The purpose of the research:
to gain new knowledge about the effect of post-weaning stress on the formation of the microbiota of the rumen of dairy calves, with or without the addition of NCG, which catalyzes the efficiency of the utilization of ammonia and other decomposition products of nitrogen-containing products.

The duration of the experiment - 30 days.
The age of calves at the beginning of the experiment - 1 month.

Group	animals in the group	Feeding characteristics
1 - control	8	The main ration (MR)
2 - testing	8	MR + 20 mg NCG per kg of live weight

Isolation of DNA from the rumen content

T-RFLP- analysis (terminal restriction fragment length polymorphism)

analysis of results

T-RFLP analysis was performed on Beckman Coulter CEQ-8000 Analyzer (USA), peak processing was performed in the Fragment Eneliziz program ("Beckman Coulter", USA) at Biotrof LLC

БИОТРОФ
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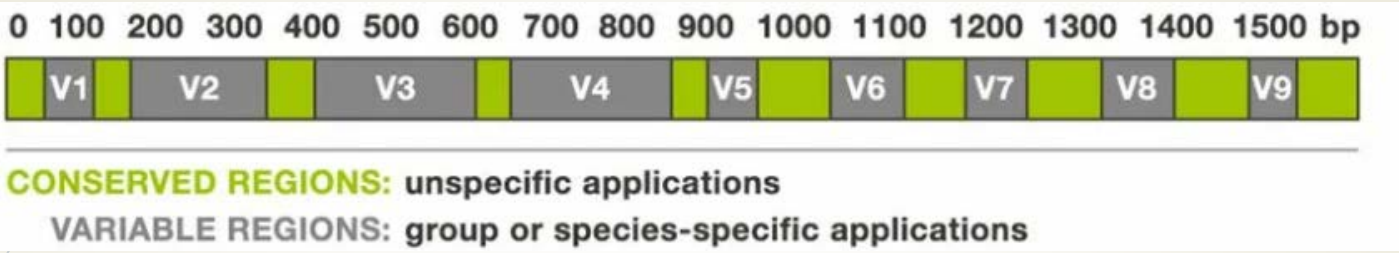
Target: 16S rRNA gene

The 16S rRNA gene sequence is used in the study of the phylogenetics of bacteria and archaea, and is used for medical studies of pathogenic bacteria.



1

PCR

2

V1V9 – amplicons, approximately 1400 bp

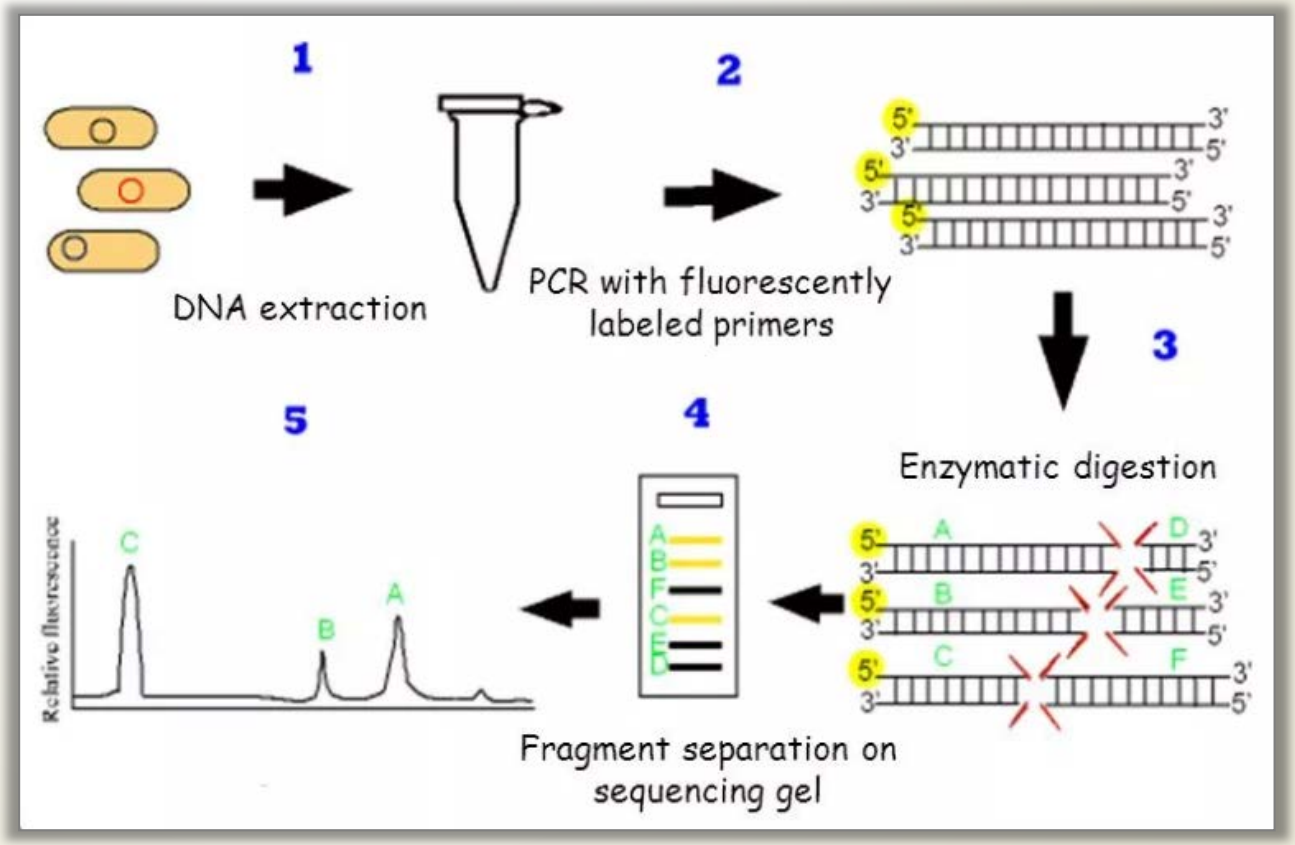
3

Restriction: *HaeIII, HhaI,MspI.*

Result:
a lot of labeled restriction fragments of different sizes

4,5

Stages of performing T-RFLP analysis



RESEARCH RESULTS

Results of weighing of dairy calves (M±m; n=8)

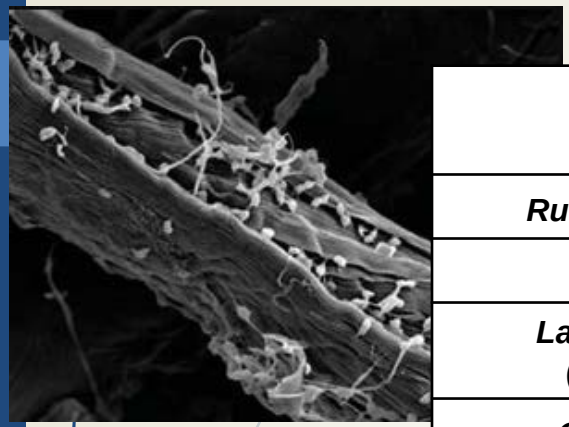
Groups	weight of calves, kg		
	before the study	after 30 days	average daily weight gain*
Control	73,4 ± 2,41	83,8 ± 3,42	0,35 ± 0,07
NCG	72,0 ± 1,34	88,0 ± 1,87	0,49 ± 0,09

* The rate of weight gain of young Holstein breed at the age of 0-2 months is 620-650 g/day

Weaning is one of the most severe stresses in the life of a calf: there is a change in the diet, temperature, and the external conditions of detention change. Weaning often coincides with the transportation of calves.

Multiple stresses arising in these new conditions of existence easily lead to various diseases of calves: respiratory, gastrointestinal - first of all

The content of the main cellulolytic bacteria in the rumen of calves of the experimental and control groups, %



Bacteria	Control	NCG	Reference values
Ruminococcaceae	4,07 ±1,22	**8,87 ±3,43***	no < 5
Eubacterium	6,84 ± 2,34	7,24 ±1,54	no < 2
Lachnospiraceae (Butyrivibrio)	3,06 ± 0,63	**4,84 ±0,75***	no < 2
Clostridiaceae	1,54 ± 0,32	**3,78 ±0,63****	no < 5
Bacteroides	2,47 ± 0,81	4,24 ±1,62	4-8%
Prevotella	0,73 ± 0,32	0,90 ±0,53	
Other	0,06 ± 0,04	0,20 ±0,18	
Total:	18,77 ± 1,54	**30,08 ±3,05****	no < 20
Cellulolytic activity,%	11,06 ± 0,35	**12,77 ± 0,71***	
Content of infusoria, thousand pcs./g	114,6 ± 1,1	**106,0 ±0,5****	

*p < 0,05;
** p < 0,01;

The content of ammonia and urea in the blood of calves

	Control	NCG	Reference values
Urea, mmol/l	4,75 ± 0,16	**3,94 ± 0,16***	3,3 – 6,7
Ammonia, μmol/l	124,76 ± 9,02	**59,10 ± 4,30***	не более 50

Conclusion

- The introduction of NCG into feed in dosages of 20 mg / kg of live weight contributes to a significant increase in the content of cellulolytic microbe of calves' rumen by almost 50% compared with the control group, reduces the content of opportunistic microbiota. The cellulolytic activity of the rumen content correlated with these indicators.
- Against the background of a high level of ammonia in the blood of calves of the control group, probably caused by an increased content of pathogenic microorganisms in calves of both groups, the use of NCG with feed allowed not only to bring the concentrations of ammonia and urea in the blood to normal values, but also to improve the biochemical and zootechnical indicators of experimental animals.
- NCG helps to increase the protection of the microbiota of the rumen and the body of dairy calves from negative stress factors, in particular, in conditions close to hyperammonemia.

Thank you for your attention