



## EFFECT OF FEED ADDITIVES BASED ON MEAT-BONE MEAL AND PROBIOTIC ON RUMEN DIGESTION PROCESSES AND SPERM QUALITY OF BREEDING RAMS

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### Abstract

The main technological process in sheep breeding during feeding and keeping breeding rams used for artificial insemination is the preparation of rams in the pre-mating period, which is 1.5-2.0 months before the artificial insemination of sheep. In this regard, the physiological state and sexual activity of breeding rams depend on the level of feeding. The study used a complex feed additive containing the probiotic "Baktovit", Premix "Novamix" and meat-bone meal. The results showed a positive effect of this supplement on the digestion processes and sperm quality of breeding rams of the Karakul breed. In the experimental group of rams, where the complex feed additive was used, the number of infusoria in 1 ml of rumen fluid was higher than in the control, both before and after feeding by 29.3 thousand or 5.11% ( $P < 0.05$ ) and 126.7 thousand or 19.25% ( $P < 0.01$ ), respectively. This contributed to an increase in the coefficient of digestibility of organic matter by 2.48% ( $P < 0.05$ ). Microscopic studies of ram sperm showed that the average volume of ejaculate and the concentration of sperm in 1 ml of sperm in the experimental group exceeded the control by 0.22 ml or 19.85% and 0.46 billion / ml or 17.5% ( $P < 0.01$ ), respectively; the resistance index was higher by 4.11 thousand or 12.5% ( $P < 0.01$ ), the respiration of the sex cells was more intense by 1.66 minutes.

**Key words:** breeding ram, feed, probiotic, premix, meat-bone meal, feed digestibility, infusoria, sperm quality.

### INTRODUCTION

Physiological functions of the body, including sexual reactions in animals, are highly dependent on the level of feeding. Adequate feeding and maintenance of breeding rams is an important factor in increasing their sexual activity and sperm quality. Breeding rams should be provided with high-yielding pastures with concentrated feed, while the rations should be balanced in terms of protein, vitamin and mineral nutrition [1; pp. 16-28], [10; pp. 8-17].

The main technological process in karakul breeding for feeding and maintaining breeding rams used for artificial insemination and free mating, as well as test rams, is year-round pasture maintenance. Important in this process is the preparation of rams in the pre-mating period, which is 1.5-2.0 months before the the artificial insemination of sheep and occurs in September and the first half of October.

The aim of the study was to assess the effect of a complex feed supplement containing the probiotic "Baktovit", Premix "Novamix" and meat-bone- meal on the digestion processes and sperm quality of breeding rams of the Karakul breed.



### RESEARCH COUDITIONS AND METHODS

The norms and rations for feeding rams were determined on the basis of reference data [3: p. -456]. The need and daily amount of eaten pasture forage on pastures by seasons of the year for Karakul sheep were determined on the basis of methodological data [4; p. -173].

The chemical composition of the feed samples was studied using the zootechnical analysis method: initial moisture content by drying the samples in a drying cabinet at a temperature of 60-65 °C and hygroscopic 100-105 °C and total moisture content by calculation; crude protein content by determining the total amount of nitrogen by the Kjeldahl method in combination with isothermal distillation; crude fat content by extracting samples in a Soxhlet apparatus using an organic solvent (disulphide ether); crude ash content by burning a sample in a muffle furnace at a temperature of 450-500 °C; nitrogen-free extractive substances (NFE) content by calculation, subtracting the moisture, protein, fat, fiber and ash content from 100% [8; p. -256].

To study the metabolism of the rumen of experimental sheep, samples of rumen fluid were extracted using a medical probe, the identification and counting of ciliates microscopically - according to the method of [12; p. -142] and [5; pp. 19-23].

To study the rumen fluid, it was extracted using a medical probe, and ciliates were identified and counted microscopically according to the method of [12; p. -142] and [5; p. 19-23].

Sperm was obtained by the urethral method, microscopic studies of the sperm of stud rams were carried out according to the recommendations of [11; pp. 58-110], where the following were determined: ejaculate volume using a measuring pipette; density by saturation of spermatozoa, motility visually by 10-point system of evaluation of spermatozoa possessing rectilinear-translational movement, concentration in 1 ml by counting in Goryaev's counting chamber, resistance by resistance of spermatozoa to 1% solution of sodium chlorine until the end of rectilinear movement, reducing capacity by duration of discoloration in methyl blue.

### RESEARCH RESULTS AND DISCUSSIONS

Determining the need and satisfaction of sheep in pasture feed is a complex process. The study used methodological and reference data from scientists at the Research Institute of Karakul Sheep Breeding and Desert Ecology, whose research can be considered classic in studying the feeding and maintenance of Karakul sheep [4, -173].

The determination of the chemical composition of pasture feed is the main indicator in determining the level of feeding of experimental animals (Table 1).

**Table 1.**

**Chemical composition and content of nutrients in pasture grass, (September)**



Indicators	In absolutely dry substance, %	Contained in food with natural moisture	
		%	г
Water	-	46,55	465,5
Dry matter	-	53,45	534,5
Crude protein	7,25	3,88	38,5
Crude fat	2,25	1,20	12,0
Crude fiber	37,64	20,12	201,2
NFE	43,2	23,09	230,9
Ash	9,66	5,16	51,6
Carotene, mg	7,0	3,2	32,0

The chemical composition of pasture grass, studied in the season under study with a dry matter content of 53.45% in the forage sample in its natural state, showed that the data on average correspond to that for the foothills represented by the ephemeroïd semi-desert.

To increase the protein nutritional value of the rams' diet, meat-bone meal was used. The study [7; pp. 39-43] notes the importance of increasing the protein nutritional value of the rams' diets, and the author claims that as a result of using enriched compound feeds in the rams' diets, the ejaculate volume increases by 5%, the sperm concentration by 8.2-14.9%, which contributes to an increase in the fertility of ewes by 8.5%. In this regard, according to the research methodology, meat-bone meal was used in the rams' diets as an additional source of protein nutrition. The chemical composition and nutrient content of meat and bone meal are given in Table 2.

**Table 2.**  
**Chemical composition and nutrient content of meat and bone meal**

Indicators	In absolutely dry substance, %	Contained in food with natural moisture	
		%	г
Water	-	9,95	99,50
Dry matter	-	90,05	900,50
Crude protein	35,27	31,76	317,61
Crude fat	22,74	20,48	204,77
Crude fiber	1,34	1,21	12,07
NFE	40,65	36,61	366,05

The data in the table show that meat-bone meal is a high-protein feed, which contains 317.6 g of protein per 1 kg with natural moisture. These indicators and the content of other nutrients were used to formulate feed rations for stud rams.

To determine the energy value of meat and bone meal, average digestibility coefficients and generally accepted energy coefficients of 1 g of PV were used (for protein - 23.3; fat - 39.7 and carbohydrates 17.5 kJ). The calculation of the energy value of meat and bone meal in exchange energy and ECU are given in Table 3.



**Table 3.****Calculation of the energy value of meat and bone meal**

Indicators	Unit of measurement.	Protein	Fat	NFE
In absolutely dry matter	%	35,27	22,74	1,34
In 1 kg of dry matter	g	352,7	227,4	13,4
Digestibility coefficient	%	79	92	50
Digestible nutrient	g	278,63	209,21	6,70
Energy coefficient	KJ	23,3	39,7	17,5
Metabolizable energy of nutrient	KJ	6492,15	8305,56	117,25
Total, metabolizable energy	KJ	14914,96		
	MJ	14,91		
Energy feed unit	EFU	1,49		

The data in Table 4. showed that the content in 1 kg of the studied meat-bone meal is 14914.96 kJ, or 14.91 MJ or 1.49 FEU. This indicator, on average, corresponds to the reference indicators [2; p. -650], (1.15 EFU). The data on the chemical composition and energy value of meat-bone meal were used in the preparation of rations for stud rams (Table 4.).

**Table 4.****Rations for stud rams**

Indicators	Pre-marital period		Mating period	
	Control group	Experimental group	Control group	Experimental group
Pasture grass, kg	3	3	-	-
Alfalfa hay, kg	-	-	1,5	1,5
Carrots, kg	0,5	0,5	0,5	0,5
Barley bran, kg	0,4	0,4	0,8	0,8
Cottonseed meal, kg	0,1	0,1	0,1	0,1
Meat and bone meal, kg	-	0,05-0,1	-	0,1
Table salt, g	15	15	15	15
Premix, g	-	5	-	10
Probiotic, g	-	3	-	-
The diet contains:				
FEU	1,7	1,8	2,1	2,2
Dry matter, kg	2,1	2,2	2,1	2,2
Digestible protein, g	141	166	256	282
Crude fat, g	47	68	53	73
Crude fiber, g	641	641	437	437
NFE, g	1019	1020	1077	1078
Carotene, mg	123	123	101	101



The main feed ration of the experimental stud rams in the pre-mating period consisted of pasture grass (determined by the calculation method) - 3 kg, carrots - 0.5 kg, barley meal - 0.4 kg, cottonseed meal - 0.1 kg and table salt - 15 g. In the second period, the basis of the diet was: alfalfa hay - 1.5 kg, carrots - 0.5 kg, cottonseed meal - 0.1 kg and salt 15 g per head per day. In these diets, the average nutritional value of the diets was 1.7 in the first and 2.1 FEU in the second feeding period, with a digestible protein content of 141 g and 256 g, respectively. It should be noted that the protein content in the diets on average corresponds to the needs of animals of a given sex and age group, physiological state, as well as the purpose of their use. However, in intensive production, increasing the protein nutritional value of diets by increasing the biological value of proteins and the content of essential acids is important in feeding producers.

As noted by [13; p. 86], for valuable breeding rams used with with intensive use, it is necessary to include skim milk (1-2 l) and raw eggs (3-5 pcs. per head per day) in the diet. Recommendations for introducing animal feed into the diet of rams to increase protein nutrition are also indicated in other studies. Considering the feed base of this farm, animal feed is not used in feeding rams to prepare for the mating period, as well as during the mating period. In this regard, the use of meat-bone meal in the diet of rams is effective for the intensification of pasture livestock farming, since this feed has a low cost price.

According to the research methodology, additional feeding was included in the diet of the experimental group. It should be noted that meat-bone meal has a specific smell, which requires accustoming animals to eating it. In the pre-mating period, rams were accustomed to gradually eating this feed, increasing the dose to 50 g, then to 100 g by the mating period. For this, the additive was thoroughly mixed with concentrates, which ensured their complete palatability. The premix additive was 5 g in the first and 10 g in the second feeding period, the probiotic was only in the pre-mating period at 3 g per head per day.

The quantitative and qualitative composition of the diet leads to corresponding changes in the forestomachs, the studies of which are reflected in many studies conducted on sheep [14; pp. 1-7], [15; pp. 378-386], [16; pp. 1181-1193], [17; 271-277]. In this regard, some indicators of rumen metabolism of rams were studied in the studies using feed additives in a complex (Table 5).

**Table 5.**

**Indicators of rumen metabolism of breeding rams,  $\bar{X} \pm S$ , (n=3)**

Group	Before feeding		3 hours after feeding	
	pH	Content of infusoria, thousand/ml	pH	Content of infusoria, thousand/ml
Control	6,80±0,04	573,0±8,57	6,72±0,05	658,0±7,97
Experimental	6,72±0,04	602,3±5,31*	6,68±0,04	784,7±9,63**

(\*-P<0,05; \*\*-P<0,01)

The data in Table 5 indicate that after feeding, active development of unicellular flora in the rumen of experimental animals is observed, a decrease in the acid-base balance of the



rumen fluid. In this case, in the experimental group, the number of infusoria in 1 ml of rumen fluid was higher than in the control, both before and after feeding by 29.3 thousand or 5.11% ( $P < 0.05$ ) and 126.7 thousand or 19.25% ( $P < 0.01$ ), respectively.

From the above data, it can be concluded that feed additives with a composition of probiotic, premix and meat-bone meal contributed to the active growth and development of ciliates in the rumen of rams.

Numerous studies on the digestibility of feed using feed additives have proven the positive effect of probiotic and vitamin-mineral supplements on the studied parameter. Unlike other experiments, in addition to the probiotic and premix, meat-bone meal was used in feeding the stud rams. As is known, animal feed, unlike plant feed, has a higher content of essential amino acids, which serve as an indicator of the completeness of the protein. In turn, complete proteins are an important factor in increasing the digestibility of feed, which serves as a scientific basis for using these types of feed in feeding farm animals.

In physiological experiments to study the digestibility of organic matter in the diet of stud rams, the Lancaster method was used (Table 6).

**Table 6.**

**Digestibility of organic substances,  $X \pm S$ , (n = 3)**

Group	Nitrogen content in feces, g	Crude protein content in feces, g	Digestibility coefficient organic matter, %
Control	1,06±0,01	6,63±0,06	62,21±0,64
Experimental	1,02±0,01	6,38±0,05	64,69±0,60*

(\*- $P < 0,05$ )

It was found that the nitrogen content in the feces of the rams in the control group was 1.06%, which corresponds to the protein content in the feces of 6.63% ( $1.06 \times 6.25$ ), while the digestibility coefficient of organic matter was 62.21%. In the experimental group, these indicators were 1.02; 6.38 and 64.69%, respectively. As can be seen, in the experimental group, the digestibility coefficient of organic matter was higher than in the control by 2.48% ( $P < 0.05$ ).

The sexual instinct in animals is one of the main ones along with the food instinct and the instinct of self-preservation, which can be assessed based on observations. The ethology of sexual reflexes in males determines their physiological potency due to hormonal changes in the endocrine system, neurohumoral status and the general state of the body's metabolism. In this regard, when preparing rams for the pre-mating period, accustoming them to an artificial vagina and assessing their sexual behavior is an important zootechnical measure in preparation for the mating period.

The quality of sperm depends greatly on the level of feeding, conditions of maintenance, health status and correct sexual exploitation of the producer.

For a comparative analysis of the quality and properties of sperm production of ram-producers between the control and experimental groups, the first 30 ejaculates were studied (Table 7). Sperm samples in all groups were assessed as thick, which indicates a normal concentration of sperm diluted in the secretion of the accessory glands. In the field of view of



the microscope eyepiece, almost complete filling with sperm was observed, between the observed objects were barely noticeable minor gaps in space. The movement of sperm in the samples was flowing or vortex with high activity, which indicated the absence of signs of oligospermia or aspermia.

**Table 7.**

**Microscopic indices of sperm of rams-producers,  $X \pm Sx$ , (n=3)**

Indicators	Unit of measurement	Control group	Experimental group
Density	score	G (all samples)	G (all samples)
Motility	score	8±0,71	9±0,71
Sperm obtained	ml	33,67±1,08	40,33±0,82*
Ejaculate volume	ml	1,12±0,04	1,34±0,03*
Average concentration	billion/ml	2,63±0,06	3,09±0,08*
Doses obtained (0.05 ml)	quantity	673±21,60	806±16,33*
Sperm count in one dose	million/0.05 ml	267±5,71	308±7,76*
Resistance	thousand	32,71±0,65	36,82±0,54*
Reducing capacity	minute	7,04±0,31	5,37±0,21*

(\*-P<0,01)

The motility score of diluted sperm in the control group was estimated at 8 points or 80% of sperm had straight-forward movements, or G-8, in the experimental group this indicator was 9 or G-9, respectively. As is known, for artificial insemination of sheep it is recommended to use only thick ram sperm and at least 8 points according to the motility score. Thus, the ram sperm obtained in the control group had minimal indicators of density and motility for use in artificial insemination. According to this indicator, the sperm obtained in the experimental group exceeded by 1 point.

For the first 30 ejaculates from one ram, an average of 33.67 ml was obtained in the control group. In the experimental group, this volume was 40.33 ml, which exceeded the control indicator by 6.67 ml or 19.8% (P<0.01). In terms of average ejaculate volume and sperm concentration in 1 ml of sperm, the experimental group exceeded the control group by 0.22 ml or 19.85% and 0.46 billion/ml or 17.5% (P<0.01), respectively.

As noted by [11; p. 58-110], during natural insemination of sheep, about 100-150 million sperm enter the cervix, which is about 3-5%. Since, in artificial insemination of sheep by the cervical method, sperm is introduced directly into the uterus or cervical canal, it is proposed to introduce as much as is introduced during natural mating. Therefore, the dose of undiluted sperm is 0.05 ml. Based on this, the calculation of the total number of doses in the experimental group was 806 and the number of sperm in one dose was 154 million, which exceeds the control according to these indicators by 133 doses or 19.8% and 23.0 million or 17.5% (P<0.01), respectively.

In the experimental group, the resistance index was higher than in the control by 4.11 thousand or 12.5% (P<0.01), which means the resistance of sperm protoplasm to the penetration of sodium ions contained in the sodium chloride solution. According to the



reducing capacity of ram sperm, or the intensity of oxygen absorption for decolorizing the methyl blue solution for 3-7 minutes, this is a good indicator [11; p. 62]. Thus, this indicator in the sperm of the control and experimental groups is good. However, the respiration of the sex cells in the experimental group was 1.66 minutes more intense.

Thus, a microscopic study of the quality of sperm of stud rams showed that according to the main indicators in all groups, the samples met the requirements for use in artificial insemination, but in the experimental group these indicators were better.

### CONCLUSION

Drawing conclusions based on the results of the experiment on feeding breeding rams, it can be concluded that the complex of feed additives with the composition of probiotic, premix and meat and bone meal in the diet of breeding rams in the pre-mating period had a stimulating effect on sexual activity and increased sperm quality, which in turn affected the fertilizing ability of breeding rams. Thus, increasing the biological value of diets is important in increasing the fertilizing ability of breeding rams.

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