IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 17, Issue 10 Ser. 1 (October. 2024), PP 15-21
www.iosrjournals.org

The Effect Of Biologically Active Substances On Improving The Quality Of Ram Semen During The Non-Breeding Season

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Abstract:
Background*: The effect of a biologically active preparation derived from cyanobacteria on improving the quality of semen in breeding rams during the non-breeding season was studied.*
Materials and Methods*: Rams in the experimental group were given an additional 7 ml of the biologically active preparation daily, along with their main diet, for 50 days.*
Results*: The introduction of the biologically active preparation derived from cyanobacteria into the diet of rams led to a significant improvement in both qualitative and quantitative semen parameters during the non-breeding season. Sperm concentration increased by 40%, ejaculate volume by 71.4%, and the total number of spermatozoa in the ejaculate increased by 140%. A significant increase in motile spermatozoa was observed, reaching 81.25%, and spermatozoa with straight-line progressive motility increased to 39.25%, indicating a substantial improvement in semen quality.*
The addition of the preparation to the diet also led to a significant increase in testosterone levels in the experimental group by 5.73% compared to the baseline level. This increase may positively affect the spermatogenesis process and improve the overall reproductive potential of the animals.
The use of the biologically active preparation significantly reduced the percentage of spermatozoa with damaged acrosomes at all stages of cryopreservation, confirming its positive effect on maintaining the structural integrity of spermatozoa. This improves the efficiency of cryopreservation and the subsequent use of sperm for insemination.
Conclusion: *The results showed that the preparation positively influences the quantitative and qualitative semen parameters obtained from the breeding rams in the experimental group.*
Key Word*: Ram, Ejaculate, Non-Breeding Season, Motility, Volume, Concentration, Acrosome.*

Date of Submission: 26-09-2024 Date of Acceptance: 06-10-2024

I. Introduction

In recent years, there has been a noticeable increase in interest in breeding various and more productive sheep breeds in the Republic of Moldova. This is of great importance for stabilizing and increasing the sheep population, which is one of the key challenges in sheep farming. Addressing this issue is directly linked to boosting the production of high-quality food products. One of the main factors contributing to the growth of sheep farming products is not only improving the productivity of the sheep themselves but also the intensive reproduction of the flock. This involves utilizing the biological potential of the breeding stock and introducing high-value rams for breeding (Miclea et al., 2005, 2008; Boettcher et al. 2010).

Under year-round use of rams, adjustments in their reproductive function may occur, making it particularly important to study ways to improve the biological integrity of sperm outside the breeding season (Epishina, 2009; Zăhan, 2017). Literature sources emphasize the importance of using biologically active substances to stimulate and regulate the physiological functions of high-value producers (Darie et al., 2020; Ayman et al., 2021). The effect of using biologically active substances is explained by their ability to activate the body's regulatory systems: enhancing regenerative capabilities, increasing nonspecific resistance, boosting catalase levels, and regulating acid-base balance. Additionally, they improve the body's reactivity and enhance the functional state of the reticuloendothelial system. These substances also promote increased immunobiological activity, stimulate gas exchange, glycolysis, phosphorus metabolism, hematopoiesis, and improve cardiovascular, respiratory, and other vital functions (Cibotaru et al., 2022; Osipchuk et al., 2023).

Among the many plant-based products with protective, therapeutic, and bioproductive effects, the biomass of cyanobacteria stands out, from which a range of bioactive substances is obtained using modern biotechnologies. (Rudic, 2007; Zinicovscaia et al., 2017).

It is well known that one of the main advantages of artificial insemination is the ability to maximize the use of the genetic potential of particularly valuable sires. This significantly accelerates the process of increasing animal productivity on breeding and commercial farms. To achieve this, it is necessary to substantially increase the offspring produced by elite sires (Bagirov et al., 2017). This can be accomplished through the use of methods that stimulate and regulate the reproductive functions of high-value sires during the off-season, allowing for the accumulation and long-term storage of semen for subsequent artificial insemination of animals (Mamontova et al., 2021). However, experimental data indicate that this method has not yet gained widespread adoption. This is because semen obtained from elite breeding rams is often unsuitable for cryopreservation and long-term storage in genetic banks.

The production of high-quality semen plays a key role in modern sheep breeding methods, as artificial insemination not only improves the animals' genotypes but also preserves their genetic heritage. Effective management of reproduction processes on breeding farms requires in-depth knowledge and constant oversight by breeders. Therefore, the genetic value of rams used in these processes is a crucial factor. In addition, the use of high-quality semen accelerates genetic progress, reduces disease incidence, and increases flock productivity, which is especially important for the sustainable development of sheep farming.

In this regard, there is a need for research focused on studying substances capable of stimulating the reproductive function of breeding rams under specific physiological and environmental conditions. This will ensure the profitability of maintaining and using these animals, especially during periods when they are not actively reproducing

II. Material And Methods

The materials of the article were prepared in accordance with project 20.80009.5107.20: "Management of the genetic potential and production of breeding animals reproduced and exploited under the pedoclimatic conditions of the Republic of Moldova," as well as project 220101: "Scientific support for the valorization of zooveterinary resources, selection and adaptation of new breeds and hybrids, harmless curative technologies and methods under climatic resilience conditions."

In the experiments conducted, clinically healthy breeding rams of the Moldovan type of the Tsigai breed were used, characterized by their wool, meat, and milk productivity. Two groups of animals were formed for the experiment, with 5 rams in each group. All animals were carefully selected and kept under identical conditions throughout the entire experiment. The rams in the experimental group were given an additional 7 ml of a biologically active preparation derived from cyanobacteria daily, along with their main diet. This preparation was specially developed and produced by the Institute of Microbiology and Biotechnology at the Technical University of Moldova. The duration of the experiment was 50 days. The research was conducted during the non-breeding season at the STE "Maximovca" Sheep Farm - Experimental and Technological Station. Semen from the rams was collected using the standard method with an artificial vagina.

At the beginning and end of the experiment, the semen was analyzed for the following parameters: ejaculate volume, sperm concentration, sperm motility, and the percentage of sperm with straight-line progressive movement. Additionally, the speed of sperm movement was evaluated based on various parameters: average path velocity (VAP), straight-line velocity (VSL), and curvilinear velocity (VCL). Moreover, testosterone levels in the animals' blood were analyzed.

The ejaculate volume was measured immediately after collection using a sterile graduated test tube.

The ram semen was diluted using a synthetic SCG medium (sucrose-citrate-egg yolk) with the following composition, % by volume: sucrose 6.4, sodium citrate 0.8, chicken egg yolk 10, glycerol 5, aqueous solution of mannan proteins 500 mg/ml at 0.5-0.7, and bidistilled water 100. The semen was frozen in pellet form.

Sperm resistance to freezing was studied by determining sperm motility after thawing.

A qualitative analysis of the semen samples was performed using the "CEROS" computer program.

The necessary statistical calculations were performed using the "Excel" program for Windows on a computer. The significance of the difference between the mean values of the compared groups was determined using Student's test.

III. Result

Experimental data on the study of the effect of a biologically active preparation derived from cyanobacteria on the dynamics of sperm concentration in the ejaculate, ejaculate volume, and the total number of spermatozoa in the ejaculate of breeding rams during the experiment are presented in Figure no 1.

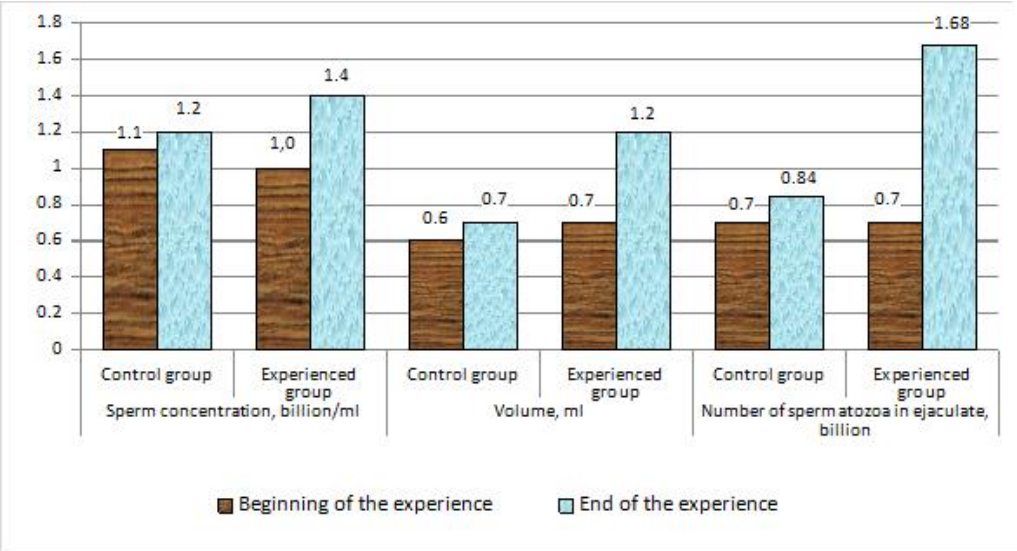

Figure no 1: Qualitative and quantitative indicators of breeding ram semen

Based on the conducted research, it was found that the average sperm concentration in the ejaculate (Figure no 1) at the beginning of the experiment was approximately the same in all groups, amounting to 1.1±0.04 - 1.0±0.03 billion/ml. At the end of the experiment (after 50 days), a significant increase in sperm concentration was observed in the experimental group, reaching 1.4±0.1 billion/ml (P≤0.05), which is 0.4 billion/ml (40%) higher compared to the initial results.

The average ejaculate volume at the beginning of the experiment was 0.6±0.07 ml in the control group and 0.7±0.06 ml in the experimental group.

By the end of the experiment, an increase in ejaculate volume was observed in the experimental groups. The increase was recorded in the group of animals that received a biologically active preparation derived from cyanobacteria. In this group, the ejaculate volume reached 1,2±0,73 ml, which is 0.5 ml (71.4%) higher compared to the initial results.

However, considering the direct correlation between ejaculate volume and sperm concentration, significant changes were observed by the end of the experiment. At the beginning of the study, the number of spermatozoa in the ejaculate across all groups was approximately the same at 0.7 billion. Fifty days after the application of the biologically active preparation derived from cyanobacteria, the number of spermatozoa in the ejaculate in the experimental group increased to 1.68 billion (P≤0.05), which is 0.98 billion (140%) higher compared to the initial results.

Additionally, sperm motility was studied throughout the experiment. The ram semen was diluted with the SCG diluent. The data on the study of motile and straight-line progressive spermatozoa in the ejaculate during the non-breeding season, depending on the use of the biologically active preparation, are presented in Figure no 2.

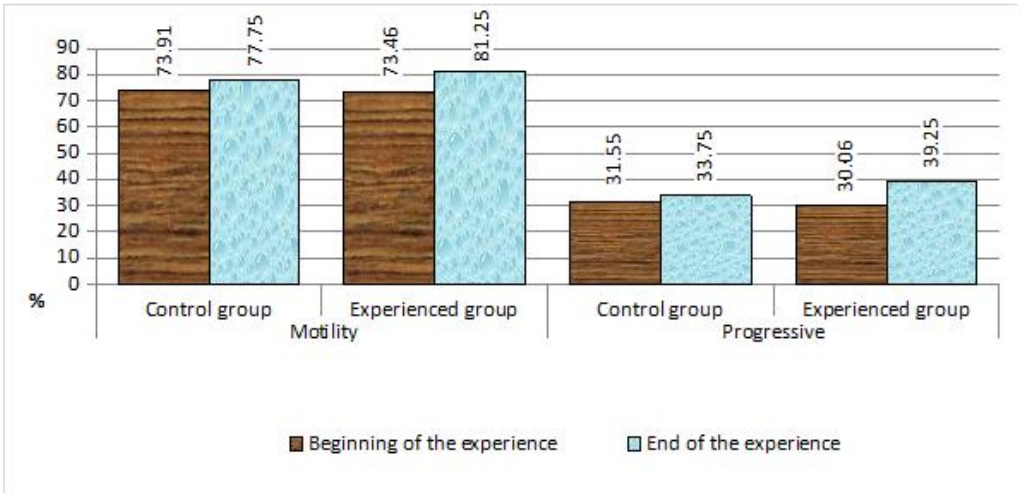

Figure no 2: Percentage of motile spermatozoa and sperm with straight-line progressive movement

Based on the obtained results (Figure no 2), the percentage of motile spermatozoa in ram ejaculate at the beginning of the experiment was 73.91±5.4% in the control group and 73.46±2.4% in the experimental group. By the end of the experiment, the percentage of motile spermatozoa in the experimental group of rams increased significantly to 81.25±1.38% (P≤0.05).

A similar trend was observed in the number of spermatozoa with straight-line progressive movement. At the beginning of the experiment, the percentage of straight-line progressive spermatozoa in the control group was 31.545±3.398%, and in the experimental group, it was 30.06±2.44%. By the end of the experiment, the number of straight-line progressive spermatozoa in the ejaculate of rams in the experimental group significantly increased compared to the initial values, reaching 39.25±3.54% (P≤0.05).

During the experiment, the effect of the biologically active preparation derived from cyanobacteria on sperm motility parameters was studied. The experimental data on the effect of the preparation on sperm motility (VAP, VSL, VCL) at the beginning and end of the experiment are presented in Figure no 3.

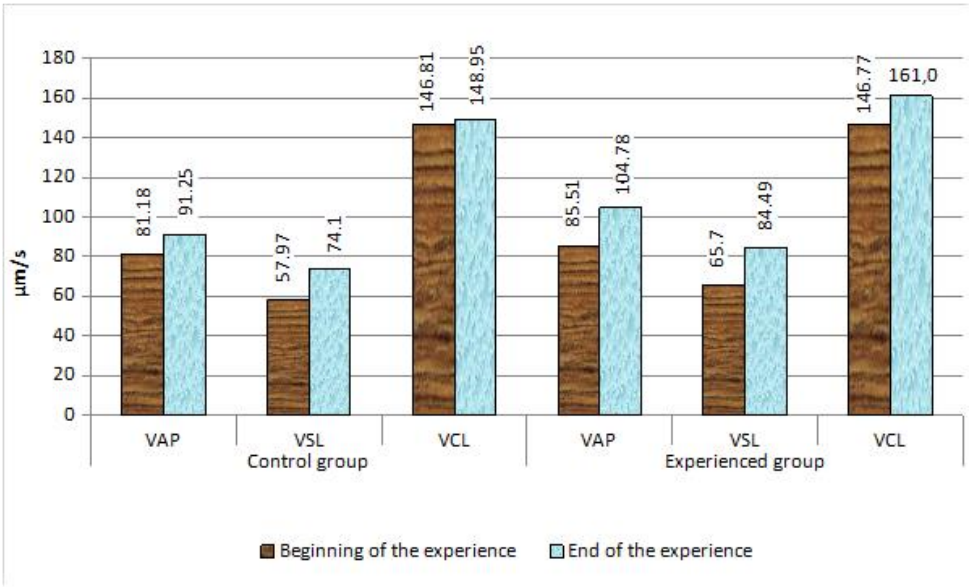

Figure no 3: Sperm motility speed (VAP, VSL, VCL)

The experimental data presented in Figure no 3 show that sperm motility speed (VAP) at the beginning of the experiment in the control group was 81.18±4.17 µm/s, VSL was 57.97±2.96 µm/s, and VCL was 146.81±8.24 µm/s, while in the experimental group, the values were 85.51±4.98 µm/s, 65.7±3.92 µm/s, and 146.77±8.95 µm/s, respectively. After 50 days of applying the biologically active preparation derived from cyanobacteria, the VAP and VSL motility speeds in the experimental group increased significantly (P≤0,01) compared to the initial values, reaching 104.78±4.6 µm/s and 84.49±5.33 µm/s, respectively. The VCL motility speed in the experimental group also increased to 161±6.319 µm/s, but this difference was not statistically significant.

Additionally, the effect of the biologically active preparation derived from cyanobacteria, introduced into the main diet, on testosterone levels in the blood of breeding rams was studied. The data reflecting the results of the research on testosterone dynamics in the blood are presented in Table no 1.

Table no 1: Testosterone levels in the blood of breeding rams

Indices	Control group		Experienced group	
	Beginning of the experience	End of the experience	Beginning of the experience	End of the experience
Testosteron (ng/ml)	4,55±0,43	6,81±0,23	4,51±0,44	7,46±0,42*
	-		5.73% higher compared to the control group	

* P≤0,05

The data presented in Table no 1 show that the testosterone dynamics, as one of the main androgens regulating the spermatogenesis process in males, had nearly identical values at the beginning of the experiment for all rams—4.5 ng/ml. The introduction of an additional component of the biologically active preparation derived from cyanobacteria into the main diet of the rams significantly increased the testosterone level in the experimental group by the end of the experiment (P≤0.05) compared to the initial level. By the end of the experiment, this value reached 7.46±0.42 ng/ml, which is 5.73% higher compared to the control group.

Research by both domestic and foreign scientists has shown that adding biologically active supplements to the main diet allows for obtaining high-quality semen from breeding rams not only during the autumn months but also in other seasons. This significantly increases the utilization rate of valuable breeders throughout the year, thanks to the ability to store frozen semen outside the natural breeding season of this type of livestock (Dadaeva et al., 2023; Rotari, 2020, Rotari et al. 2023).

The experimental data on semen quality analysis obtained from rams during the non-breeding period after equilibration at a temperature of +2 to +4 °C are presented in Table no 2.

Table no 2: Quality of ram semen subjected to cryopreservation protocol after equilibration

Indices		Motility, %	Progressive, %	Sperm motility speed, µm/s		
				VAP	VSL	VCL
Control group	Beginning of the experience	71,2±4,2	30,0±3,1	82,4±3,3	57,8±2,2	146,6±6,6
	End of the experience	68,3±2,9	33,0±3,8	90,9±6,5	72,9±5,5	141,0±6,4
Experienced group	Beginning of the experience	73,5±2,4	30,1±2,5	84,7±5,9	64,1±4,4	147,0±10,7
	End of the experience	80,6±1,4**	38,5±3,0	101,2±5,5	82,7±6,1	151,5±9,3

** P≤0,01

The experimental data presented in Table no 2 show that semen obtained from rams in both the control and experimental groups, subjected to equilibration at a temperature of +2-+4°C, underwent qualitative changes. Motility indicators showed lower values compared to fresh-diluted semen.

After equilibration, significant improvements in all key parameters were observed in the experimental group by the end of the experiment. Sperm motility reached 80.6±1.4%, with statistically significant results (P≤0.01) and a 9.7% increase in motility. The percentage of spermatozoa with straight-line progressive movement was 38.5±3.0%, showing a 27.9% increase.

Similar changes occurred in sperm motility speed during the equilibration period of semen subjected to the cryopreservation protocol. There was an increase in motility speed: VAP by 19.5%, VSL by 29.0%, and VCL by 3.1%.

The experimental data on the quality analysis of semen obtained from rams during the non-breeding season after thawing are presented in Table no 3.

Table no 3: Quality of semen subjected to cryopreservation protocol after thawing

Indices		Motility, %	Progressive, %	Sperm motility speed, µm/s		
				VAP	VSL	VCL
Control group	Beginning of the experience	39,9±2,6	16,6±1,9	78,3±4,6	62,1±4,9	114,7±6,0
	End of the experience	31,9±1,9	17,4±1,3	91,6±5,9	81,1±5,9	125,3±6,4
Experienced group	Beginning of the experience	42,6±2,2	17,6±1,9	79,0±3,6	65,1±3,4	124,9±4,4
	End of the experience	49,7±1,8***	25,9±1,1**	94,1±4,7	82,8±5,1	133,5±7,8

** P≤0,01;*** P≤0,001

The cryopreservation process significantly reduced the semen quality parameters (Table no 3). After thawing, sperm motility in the experimental group reached 49.7±1.8% (P≤0.01), which is 7.1% higher compared to the motility of sperm from ejaculates collected at the beginning of the experiment and 7.8% higher compared to the control group. The percentage of sperm with straight-line movement in the ejaculates collected from the rams in the experimental group at the end of the experiment was 25.9±1.1% (P≤0.001), or 8.9% higher compared to the control group.

The average path velocity (VAP) in the experimental group was 94.1±4.7 µm/s, which is 2.5 µm/s higher compared to the control group. The straight-line velocity (VSL) at the end of the experiment in the ejaculates collected from the experimental group rams was 82.8±5.1 µm/s, or 1.7 µm/s higher compared to the control group. The curvilinear velocity (VCL) was 133.5±7.8 µm/s in the ejaculates from the experimental group, or 8.2 µm/s higher compared to the control group, where this indicator was 125.3±6.4 µm/s.

These changes indicate that adding a biologically active preparation derived from cyanobacteria to the main diet of rams had a positive effect on semen quality. These changes are also supported by statistically significant results, making them particularly important for practical application.

The effect of the biologically active preparation on the number of sperm with damaged acrosomes in cryopreserved ram semen is presented in Table no 4.

Table no 4: Sperm with damaged acrosome, %

Groups	After dilution:		After equilibration		After defrosting	
	Beginning of the experience	End of the experience	Beginning of the experience	End of the experience	Beginning of the experience	End of the experience
Control	5,2±0,4	5,0±0,5	5,6±0,4	5,9±0,5	26,2±1,0	26,0±0,8
Experiencedl	5,3±0,5	4,3±0,6	5,7±0,4	4,9±0,4	26,1±0,9	25,2±1,0

Analyzing the experimental data (Table no 4) on the effect of the biologically active preparation derived from cyanobacteria on the cryopreservation protocol of ram semen, we find that the process of semen dilution and equilibration did not reveal significant differences between the experimental groups in terms of the percentage of sperm with damaged acrosomes. However, the cryopreservation and reanimation process led to significant changes in the percentage of sperm with damaged acrosomes in the experimental group.

In the experimental group, the percentage of sperm with damaged acrosomes at the end of the experiment was 25.2±1.0%, which is 3.17% lower compared to the control group, where this indicator was 26.0±0.8%.

IV. Conclusion

- Based on the conducted research, it can be noted that the inclusion of a biologically active preparation derived from cyanobacteria in the diet stimulates spermatogenesis processes, significantly increasing the qualitative and quantitative parameters of semen obtained from breeding rams during the non-breeding season. There was a 40% increase in sperm concentration compared to the beginning of the experiment, with ejaculate volume significantly increasing by 71.4% (+0.5 ml). Taking into account the increase in ejaculate volume and sperm concentration, the total number of spermatozoa in the experimental group increased by 140% (+0.98 billion). There was a significant increase in motile spermatozoa to 81.25% (+7.79%). The percentage of spermatozoa with straight-line progressive movement increased to 39.25% (+9.19%), which also confirms a significant improvement in semen parameters in the experimental group.
- The introduction of the biologically active preparation derived from cyanobacteria into the diet of rams led to an increase in testosterone levels in the experimental group by 5.73% (+7.46 ng/ml), which was significantly higher compared to the initial level. This could positively affect spermatogenesis and the overall reproductive capacity of the animals.
- The use of the biologically active preparation significantly reduced the percentage of spermatozoa with damaged acrosomes at all stages of cryopreservation. This demonstrates the positive effect of the preparation on maintaining the structural integrity of spermatozoa, which could improve the effectiveness of cryopreservation and the subsequent use of sperm for insemination.
- Therefore, the biologically active preparation derived from cyanobacteria that we used can be recommended for application.

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