New approaches in the study and adjustment of sperm quality in breeding roosters

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Abstract. The article describes the results of studies of rooster sperm production using the latest methods of study, previously not used on the bird to diagnose sperm defects. In the production of poultry meat, the very important criterion of success is the productivity and health of the parent flock. The number one challenge for broiler production is to get a broiler chicken from a fertilized hatching egg.

1. Introduction
Ensuring food security and full import substitution of poultry meat has been achieved in the Sverdlovsk region due to the intensification of production and the use of highly productive bird’s crosses. The body of the rooster producers is very sensitive and often fails[1, 2]. The quality of the sperm production and the fertility of the hatching egg decrease, which subsequently leads to significant losses for the enterprise [3, 4]. Sperm test methods used in poultry farms have low informativity and do not provide a deep assessment of the processes occurring in the body of roosters. To this end, we used a new ultrastructural method for studying spermatoids to determine the root causes of violations [5, 6].

When the reproductive ability of cocks decreases, they are rejected and replaced by young ones. Rotation in some herds can reach 100% of the livestock, which leads to a decrease in the profitability of production. The prolongation of reproductive performance of breeding roosters and improving the quality of sperm are very topical issues, since many enterprises are equipped with high-value imported crosses.

2. Materials and methods
As a correctional therapy, we applied nature-like technologies. For the first time, research has been conducted on breeding roosters on the use of probiotic based on Bacillus subtilis bacteria (strain 945 (B-5225), at the leading poultry farms of the Sverdlovsk region ("Reftinskaya Poultry Farm" and "Poultry Farm "Sredneuralskaya"). During the study, in order to monitor the situation, two groups were formed (experimental and control). The experimental group of roosters took a probiotic preparation based on Bacillus subtilis bacteria in the amount of 5 ml per 100 animals, according to the schedule 10-days regimen / 10-days break, starting from the 340th day of life and until the end of exploitation of the bird (the 400th day).

Indicators studied: spermogram (GOST 32277—2013 Reproductive media. Sperm. Test methods for physical properties and biological, biochemical, morphological analyzes); microbiological research.
(GOST 32198-2013 Reproduction equipment. Sperm. Methods of microbiological analysis); the method of quantitative electron microscopic examination of sperma (in accordance with the WHO Guidelines, 2010) allows a qualitative and quantitative assessment of cellular organelles necessary for the sperma to perform a fertilizing function (acrosome, nucleus, flagellum, see diagram). This method also allows identifying the cause of their functional disorders. Ultrathin sperm sections with an increase of 4500 - 25000 are prepared for the study, fixed and poured into epoxy resin. Morphological studies were carried out according to standard techniques on the 400th day of age, the bird was selected by random sampling. Sperm samples were taken on the 340th day for monitoring studies, on the 370th day sperm was taken in the experimental and control groups. Additionally, the fertilization of hatching eggs and hatchability of broiler chickens for the entire period of keeping the poultry were taken into account.

3. Monitoring and investigation of the probiotic preparation effect based on the bacteria Bacillus subtilis on the qualitative and quantitative indicators of rooster sperm and the fertility of the hatching egg

Despite the full level of feeding poultry of parental flocks, enterprises maintain a high percentage of rooster rotation about 25-50%. In different batches the percentage of culling and replacement of roosters is about 70% of the flock, due to reduced reproductive function. This phenomenon can be associated with several factors in which the main role can be given to the quality of compound feed. Rooster manufacturers do not prepare special rations and feed them with unsuitable food. It should be considered that roosters need food with a higher content of protein and a different Ca: P ratio (1:1) [1,5]. For this reason, poultry farms were interested in testing this drug on a large population to solve existing problems.

According to the results of monitoring studies of sperm products, it was found that 60% of the sperm cells have a head defect, but according to the standard, the defect should not exceed 5%. Rooster spermatooza can maintain their vitality and fertilizing ability in the female genital tract from 7 to 14 days. According to the results of studies, the viability of rooster sperm within 3 hours decreased to 50% at a rate of 70%. Normal sperm in the volume of the ejaculate was 40%, with a normal rate, this figure should be 90%.

The results of the monitoring studies revealed significant deviations in the sperm production of roosters for the main production-related indicators.

Microbiological studies of sperm during monitoring studies revealed that conditionally pathogenic microflora was found in all producing roosters: Stenotroph.meltophilia (100%) and Candidasp (80%) at a rate of no more than 10%.

During the study of the effect of the probiotic preparation, ultrastructural studies of sperm were conducted. According to the results of electron microscopy, a number of deviations were detected in sperm samples of cocks of the control group. In the field of view there were numerous defective sperm. Many spermatoozoids showed mitochondrial swelling, matrix clearing, partial or complete destruction of the cristae. Single sperm with double flagella were detected. Single spermatogenesis cells and cells with impaired differentiation were found in a state of pronounced destruction. In such cells, cytoplasm vacuolization, chromatin granularity and flagella differentiation are observed. These sperm with enlarged necks cannot penetrate through the mucous membrane and the wall of the egg, which leads to a decrease in the fertilizing ability of the cell. Due to the destruction of mitochondria in the sperm of the control group, the amplitude and speed of movement of spermatoozoids is violated, since mitochondria are the major energy cell organelles (Figure 1a).

The main cause of damage (alteration) of mitochondria is associated with impaired production of ATP. In the samples of the control group, a second type of mitochondrial swelling was revealed - with large amplitude of swelling, resulting from an increase in the permeability of the inner membrane. The result is the smoothing and fragmentation of the cristae. Large amplitude swelling can be corrected by increasing the concentration of ATP and magnesia, after damage of the outer membrane it becomes irreversible quickly (i.e. fatal). It is accompanied in vivo by the death of granules of the mitochondrial matrix, which first become enlightened, then begin to harden and flake in the inner chamber. The final
stage of death is characterized by the fact that both the inner and outer membranes are broken (Figure 1b).

**Figure 1.** The microstructure of sperm of control group roosters: (a) longitudinal sections through the middle sections of flagella. Mitochondrial swelling, partial destruction of the cristae, enlightenment of the mitochondrial matrix (×18000); (b) transverse sections of the necks of sperm at the level of centrioles. Mitochondrial swelling. Rupture of the inner and outer membrane (×56000).

In the samples of the experimental group, no abnormalities were detected (Figure 2). The samples showed an abundance of sperm. Sperm heads are large, elongated. Chromatin heads are mature, condensed, and homogeneous. Single sperm with unfused chromatin were detected. The structures of centrioles are without features. The axonema has a typical ultrastructure at different levels of the flagellum. In cells with a cytoplasmic droplet, the flagellum is formed correctly. In the majority of spermatozoa, mitochondria are of normal size, cristae are clear, well visualized.

The results of electron microscopy indicate a high impact of the probiotic preparation based on the bacteria Bacillus subtilis. It contributed to the normalization of the state of the reproductive system, having a positive effect on the structure of spermatozoa, on their qualitative and quantitative composition.

**Figure 2.** The microstructure of sperm in the experimental group: a – fragment of the sperm head. Acrosome. The sperm neck (×22000); b – the cross section of the sperm neck at the level of centrioles. Centriole has a typical ultrastructure which is composed of 9 triplets (×56000).

According to the results of the anatomical cutting, it was found that the roosters of the control group had swellings throughout the intestine, numerous adhesions in the duodenum, pancreatic deformity, which indicates digestive disorders and a decrease in nutrient supply to the cells of organs, in particular, in the testicles. Malnutrition has led to the hypotrophy of the testicles and the oppression of their functions; they are much smaller in comparison with the testicles of the experimental group. This phenomenon is due to physiological and survival processes, with a shortage of nutrients intake of poultry, all internal resources to spend on the maintenance of life, reproduction processes are inhibited, chickens stop carrying eggs, sexual activity in roosters is reduced. The fact of underdevelopment and necrotic phenomenon in the testicles is recorded in the control group (Figure 3 a).

In the experimental group the result was the opposite, the testicles were well developed, the state of the intestine, pancreas corresponded to the physiological norm (Figure 3b).
Figure 3. The state of the intestines and testicles of roosters at the age of 400 days: (a) the control group: intestinal distention, adhesions to the duodenum, pancreatic deformity. Necrotic phenomena in the testicles; (b) the experimental group. Intestines, pancreas and testicles are within the physiological norm.

The normal physiological state of the digestive tract organs and testicles in the experimental group confirms the positive influence of the probiotic preparation based on the Bacillus subtilis bacteria on the reproductive function.

In the experimental group, where the roosters received the probiotic preparation, the chickens had higher fertilization rate of the incubation eggs and the chickens yield. (Table 1).

Table 1. Results of the production tests on the use of probiotic preparation based on bacteria Bacillus subtilis on roosters

| Indicators                  | Units of measure | Regulatory indicators | The control group | The experimental group |
|-----------------------------|------------------|-----------------------|-------------------|-----------------------|
| Livestock in the housing    | heads            | 6 750                 | 7 131             | 7 352                 |
| Hatching egg                | %                | at least 98.0         | 97.0              | 98.1                  |
| Healthy young growth         | %                | at least 79.0         | 77.7              | 78.7                  |

The digital material of table 1 confirms the identified patterns. The use of a probiotic preparation for roosters according to the scheme contributed to an increase in the yield of incubation eggs by 1.1% and the output of healthy conditioned broiler chickens by 1%, which significantly affects the increase in profitability.

During the production tests, the economic effect of the use of a probiotic supplement based on bacteria Bacillus subtilis was calculated. The cost of upgrading part of the herd is reduced, due to the fact that it is not necessary to replace part of the party of old roosters with young roosters in 2 months before the end of the end of the life. To grow one rooster costs about 512.85 rubles, replacement of 30% of chicken flock costs 205,000 rubles, and the cost of a probiotic preparation for one rooster for the entire period of operation costs 10.50 rubles. The economic effect of the use of the probiotic preparation was 13.67 rubles per 1 ruble invested.

4. Conclusion
The obtained experimental material on a set of indicators confirmed the positive impact of the probiotic preparation based on Bacillus subtilis. Normalization of metabolic processes in the body, strengthening the functioning of the reproductive system and improving the quality indicators of sperm demonstrate
the feasibility of using this drug. The use of probiotic preparations according to the proposed scheme will increase the economic effect and reduce the cost of maintaining breeding roosters for breeding plants, reproducers and industrial poultry farms, by reducing the rotation (replacement) of the livestock by 25-50%, and also by increasing the fertility of hatching eggs and hatchability of chickens.

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The significance of the results is confirmed by the acts of implementation and positive application for the invention reg. No. 2016126825.

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