

Biologically Active Preparation and Reproductive Function of Stud Rams

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Abstract

Aims: This research work is aimed at studying the physico-chemical properties of the apistimul preparation created on the basis of drone homogenate and sodium chloride lyophilized by vacuum freeze-drying and studying the influence of this preparation on the productivity and the reproductive function of stud rams. **Methods:** The study uses the above-mentioned methods for determining physico-chemical properties of the preparation, quantitative and qualitative analysis for hormones, studying the quantity and quality of sperm of stud rams, some hematological characteristics of blood, fertility of ewes, and the offspring yield. **Results:** As a result of the studies, the physico-chemical composition of the preparation was determined, and the presence of biologically active substances and hormones – estradiol, testosterone, progesterone, cortisol, and prolactin – was established. Feeding the drug to animals increased the amount of hemoglobin and erythrocytes in the blood of experimental animals, improved the qualitative and quantitative characteristics of the ejaculate, and had a stimulating effect on the reproductive function of rams. **Conclusion:** When the apistimul preparation had been used as the source of medicinal and medical products, high-quality ejaculates were obtained that were inherent to healthy stud rams, and the expected effects of increasing resistance and reproductive function in animals were obtained.

Key words: Breeding efficiency, brood, drone, ejaculate, fertility, hormones, offspring, preparation, rams, studs

INTRODUCTION

Analysis of the works of Harman,^[1] Yatsunami and Echigo,^[2] Lipinski,^[3] and Fang^[4] shows that, since the end of the 20th century, beehive products have been more widely used in modern medicine as a curative and preventive remedy. According to Mbaya^[5] and Meerson,^[6] honey, pollen, royal jelly, and propolis are unique natural products that contain balanced combinations of most important biologically active components, which determine a wide range of their medicinal properties. These products of bee-breeding have relatively high therapeutic activity, softer action on the physiological parameters of the organism, as compared to synthetic drugs; they are also safer and relatively inexpensive.

Studies of Morara^[7] and Schmidt and Schmidt^[8] show that, in a number of biologically active beehive products, royal jelly is the most important one. Schmidt,^[9] Zoltowska *et al.*,^[10] Bonomi,^[11] and Burmistrov^[12] believe that the brood, due to its unique composition, has a tonic and immunomodulatory effect on the organism, stimulates metabolism, restores activity of

the endocrine glands, improves resistance to infections and intense physical stress, and has been successfully used for treating many diseases.

Currently, all living creatures are experiencing severe loads, which affects the general physiological status, and is reflected in the shift of the ontogenetic patterns of living organisms' development. This has been shown by the clinical and physiological condition of the reproductive organs and their function, which indicates the health of animals and quality of their sperm products, which indirectly affects the amount of obtained products.

Given the relatively high production and technological pressure on the organisms of animals, searching for ways of reducing the negative factors that are harmful for the reproductive function is a very important issue. It should be

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Received: 24-03-2017

Revised: 03-06-2017

Accepted: 12-06-2017

noted that stress-generating situations cannot be prevented in the conditions of modern livestock breeding. These are the processes associated with growth, development, and functioning of genitals, changes in the type of feeding, regrouping, transportation of animals, hygienic and environmental conditions, and many others. According to Collison,^[13] the only economically and biologically beneficial method of neutralizing the negative effects of stress-generating factors is supporting the productive health with adaptogens.

Studies of Prokhod^[14] and Safonovskaya^[15] show that, in the recent years, more attention has been paid in veterinary medicine in using environmentally safe preparations of natural origin with high bioavailability, without side-effects and addiction, for the purpose of normalizing the metabolic processes and strengthening the immune system of animals.

The drone brood, according to Ilieshiu,^[16] is the closest to royal jelly in many ways, and during the active period of the season, it is present in all bee families in sufficient amount. According to Jannuzzi,^[17] Zhouv *et al.*,^[18] Caron,^[19] and Klishina,^[20] royal jelly has long been used in many countries as a component of nutrition and a medicine for people. Pharmacotoxicological assessment of the preparation by Lugansky^[21] provides an assumption about the possibility of using royal jelly and drone jelly in animal breeding. It is quite likely that introducing drone brood into domestic veterinary practice after systematic scientific analysis of its composition and biomedical properties may compensate for insufficient consumption of royal jelly, extend the capabilities of apitherapy in animal breeding, and improve reproductive and productive qualities of animals.

Considering the works of some researchers^[22-24] about using homogenates of bee broods for medicinal purposes for treating human diseases and their advice about the prospects of using drone brood with a rich complex of biologically active substances, several experiments with stud rams have been performed.

It should be noted that bee-based preparations have not found proper use as a source of medicinal and medical products in the livestock breeding industry. Sometimes, for the reason of various violations, high-value breeding animals retire early, falling short of the money spent on purchasing and growing them. Special condition is required for recovery of frequently encountered in production types of impotence state such as long-term refusal from mating with females, ejaculation of poor-quality sperm, aggressiveness of animals in the riding hall, passive behavior, and sluggish movement that reduce sexual activity of the animals.

As a physiologically efficient adaptogen and a therapeutic product for revival and improving sperm productivity of stud rams, we used the apistimul preparation based on drone-brood homogenate and sodium chloride.

During mating, stud rams have increased the need for energy and nutrients, deficiency of which worsens their state and quality of sperm. This process is especially evident when the mating campaign is undertaken in the conditions of the pasture, and the forage ration is monotonous and unbalanced for certain elements. Therefore, primary therapy and treatment of the reproductive ability of breeding agricultural animals, worsened sperm quality, sperm pathology combined with dysfunction of testes in the conditions of excessive loads, and unfavorable environment make the search for new efficient biologically active agents for improving spermatogenesis in testes and activation of the sexual function very urgent.

It is known that introduction of biologically active substances into the organism makes it possible in some way to regulate and monitor activity of many systems of the organism, including functional activity of cells in the testes and the reproductive function of agricultural breeding animals.

This work is aimed at creating a preparation based on drone-brood homogenate and sodium chloride, and the possibility of using it as a stimulating and therapeutic preparation for treating sexual dysfunction of valuable stud rams.

MATERIALS AND METHODS

This experimental work was performed between 2015 and 2017. In the study, available drone brood harvested in the apiaries in different periods of the beekeeping season and stud rams in the age range of 1.5-2 and 2.5-3 years grown in the South Kazakhstan region were used. For experiments, 4 groups of stud rams, six heads in each group, were selected, based on the principle of analogs, the fatness of the animals was average; they featured strong body build and belonged to the elite class. The main data about the experiments are shown in Table 1.

The experimental animals were fed in accordance with detailed feeding rules by Kalashnikova *et al.*^[25] The difference was that rams in experimental Groups I, II, and III received the apistimul preparation in their daily diet in the amount of 10, 15, 20 mg/kg since the beginning of preparation for mating (40 days), during the period of use (30 days), and during insemination (25 days). During the experiment, all animals were kept in identical conditions and enjoyed daily walks.

The apistimul preparation was made of fresh drone brood taken to the laboratory, where homogenate was prepared according to the methods of Safonovskaya^[15] and Budnikova.^[26]

The preparation was made adding ready chemical powder of sodium chloride to the homogenate drone-brood at the ratio of 4:1. The lyophilized powder was prepared by vacuum freeze-drying to the temperature of -5°C , under residual

Table 1: The setup of the experiment for studying the influence of the apistimul preparation

Group	Number of animals, heads	Characteristics of feeding	Added dosage, mg/kg
Reference	6	Basic diet (BD)	-
I	6	BD + Apistimul	10
II	6	BD + Apistimul	15
III	6	BD + Apistimul	20

pressure of 0.03 mmHg for 24 h, after which it was pelletized at the constant pressure of 150 MPa.

The physico-chemical characteristics of the finished product were determined by the method defined in GOST 28888-90 "Bee royal jelly":

- Organoleptic properties (appearance, color, smell, taste) – visually and by sampling;
- Mass fraction of water (%) - refractometrically;
- Mass fraction of crude protein (%) - by the Kjeldahl method for total nitrogen;
- Mass fraction of decenoic acid (%) - by the alkalimetric method, after their consequential emission with ether;
- Concentration of hydrogen ions (pH) - potentiometrically on a pH meter;
- The content of sulfhydryl groups - by amperometric titration by the method of Rustenov;^[27]
- Sex hormones (testosterone, progesterone, prolactin, estradiol, cortisol) by radioimmune methods with the use of standard kits. The calculation was made for 100 g of the product.
- The quantity and quality of sperm were studied using the methods of Goncharov *et al.*;^[28]
- Density and mobility were determined visually using a microscope;
- Mobility of male germ cells was assessed by the 10-point system;
- Concentration of male germ cells in the ejaculate (billion/ml) was determined by counting the spermatozoa in a Goryaev's counting chamber;
- Resistibility (thousands) – resistance of spermatozoa to 1% sodium chloride solution was determined by the method of Mikhailov *et al.*;^[29]
- Also, the fertilizing capacity of semen of rams was studied. For this purpose, an insemination experiment was made. Insemination was made with fresh undiluted sperm at the dosage of 0.05 ml twice per one heat. In the spring, according to the result of kidding, fertility and fecundity of ewes were determined.

We used the generally accepted biological and special veterinary and zootechnical methods of evaluating blood, quality of ejaculates, physico-chemical and organoleptic methods of studying properties of raw materials, apico-additives, beehive products, and products enriched with them, etc.

To characterize the physiological state, and for more detailed comparative evaluation of rams, some blood parameters were

determined: Percentage of hemoglobin and erythrocyte and leukocyte count. Blood for hematological studies was taken from the jugular vein into chemically clean test tubes from three animals in each group, in the morning on the day of the study, before feeding, in accordance with the general rules of aseptics. The blood was studied in a biochemical laboratory.

RESULTS

The distinctive feature of manifestations of biological activity of beehive products underlying their therapeutic properties is defined by their physico-chemical parameters and biochemical composition. Therefore, at the initial stage, the apistimul preparation, its most important physico-chemical characteristics, and contents of the main chemical groups and compounds were studied. During the very short period of ontogenesis, larvae of open bee brood accumulate large balanced stock of nutrients that help forming imagos from the egg. As a result, a complex of substances of vegetable and animal origin – beehive products – is formed. It has unique nutritional and immunomodulating properties, which allows considering them as essential components of apiology, a new kind of stimulating and therapeutic and prophylactic product of genetically valuable animals. Along with protein ($50.1\% \pm 1.0\%$) and reducing sugars ($27.8\% \pm 1.2\%$), the product contains hormones that actively influence sexual function in animals that are shown in Table 2.

Table 2 shows that the preparation contains a considerable amount of estradiol ($1004.36 \pm 45/1$ nmol/l), a slightly lower amount of testosterone (4.56 ± 0.3 nmol/l), progesterone (81.22 ± 1.5 nmol/l), cortisol (331.4 ± 10.1 nmol/l), and prolactin (798.5 ± 7.3 nmol/l). Availability of many basic biologically active substances in the preparation makes it possible to use it as a stimulating and therapeutic preparation.

In production, high-value and highly productive breeding animals fail first, as a rule. In the search for the ways of using drone-brood homogenate, problems occur with preserving it. According to Mashenkov,^[30] drone brood homogenate loses its biologically active properties when stored at the temperatures above zero. With this regard, in the experiment, we used a lyophilized preparation of drone-brood homogenate that was called apistimul.

By its organoleptic characteristics, the analyzed preparation called apistimul is a yellowish pill or pellet with a spicy

Table 2: Main physico-chemical characteristics of the apistimul preparation

Indicators	Apistimul
Mass fraction of dry substances, %	94.0±4.2
Concentration of hydrogen ions, pH <i>t</i> =19°C	6.5±0.1
Mass fraction of protein, %	50.1±1.0
Mass fraction of decenoic acids, %	7.6±1.2
Reducing sugars, %	27.8±1.2
Sulfhydryl groups, %	1098.6±20.3
Testosterone, nmol/l	4.56±0.3
Progesterone, nmol/l	81.22±1.5
Estradiol, nmol/l	1004.36±45.1
Prolactin, nmol/l	798.5±7.3
Cortisol, nmol/l	331.4±10.1

aroma. The biologically active preparation may be stored in dark containers at a room temperature of 20-25°C for up to 2 years.

Chemical analysis of the main physico-chemical parameters of the apistimul preparation gave reason to study their impact on reproductive and fertilizing capacity of breeding males.

To study the effect of the preparation on some hematological parameters, an analysis of the main indicators of blood was made in the reference and experimental stud rams. The results are shown in Table 3.

Red blood cells that participate in transferring nutrients are most important in the blood. According to Georgievsky,^[31] the physiological normal content in blood for sheep is 8.6-12.8 g/% of hemoglobin, 8-16 million/mm³ of red blood cells, and 6.0-14.0 thousand/mm³ of leukocytes.

Blood characteristics of animals in all groups were within the physiological norm. However, there was some difference between the groups. Also, there is a tendency to increasing the content of hemoglobin in the blood of rams in the experimental groups (from 9.01 to 12.07 g/l), as compared with the reference group. Working together, the unique structure of drone-brood homogenate and erythrocytes promotes the enhanced delivery of available biologically active substances to all functioning systems, including genitals of males, positively influences hematopoietic organs, and has a regulating effect on the tone of the vascular system and on the intensity of blood circulation.

When the animals received various dosages of the preparation, the content of leukocytes in blood was 6.21 ± 0.1 to 7.71 ± 0.01.

The amount and quality of sperm and its fertilizing capacity are the main indicators for assessing the efficiency of feeding

stud rams. Analysis of the results of studying the influence of the apistimul preparation on the reproductive ability and on the quality of sperm of stud rams showed that, over the entire period of preparing rams for mating, the volume of ejaculate in all groups during the period of usage increased and reached 1.30 in the first group, 1.37 in the second, 1.32 in the third group, and 1.18 in the reference group [Table 4].

The results showed that, during the final period, the highest volume of ejaculate was obtained in experimental Group II, where the volume increased by 0.32 ml, or by 30.4%, as compared to the preparatory period. In the first group, this value was 26.2%; in the third - 26.9% and in the reference group - 16.8%.

Another important indicator of sperm quality is its concentration, which during the preparatory period was almost identical in all groups, and amounted to 3.15 ± 0.1-3.20 ± 0.1 billion/ml. Compared to the preparatory period, during the period of usage, the concentration of sperm cells increased in the reference group by 12.6%, in experimental Group I - by 13.3%, in experimental Group II - by 14.3%, and in experimental Group III - by 13.6%.

Increasing the feeding norm of the preparation had a positive influence on mobility and resistibility of sperm. Compared to the preparatory period, during the period of use, mobility of sperm in all rams was higher than 8 points and 8.1 in the reference group; in experimental Group I - 8.5, in experimental Group II - 8.5, and in experimental Group III - 8.4. During the final period, the highest activity of spermatozoa was observed in experimental Group II - 9.2 points and in the reference group - 8.5.

Thus, in the period of usage, as compared to the preparatory period, in the experimental groups, sperm resistivity increased by 15.1% in the reference group, and by 19.3%, 24.4%, 18.4% in I, II, and III experimental groups, respectively. Compared to the preparatory period, semen resistivity during the final period continued to grow and achieved 25% in the reference group, and 27.9%, 31.4%, and 29.7% in the I, II, and III experimental groups, respectively.

To determine the effect of sperm quality on fertility of ewes and the offspring, on an average, 40 heads of elite females from each group were taken. The sheep were in equivalent feed conditions. The ewes were inseminated once, in the morning, with stud rams mating 2-3 times per day. Table 5 shows the results of insemination of ewes. The rate of ewes' fertilization after first insemination was 133 heads, or 79.2%. In the reference group, it was 75.5%, in experimental Groups I, II, and III, it was 78.5%, 82.5%, and 80.5%, respectively.

On an average, in the reference group, 106.2 lambs were obtained; in experimental Group I - 113, in experimental Groups II and III - 116.7 and 113.3%, respectively.

Table 3: Hematological parameters of animals' blood

Indicators	Preparatory period	Period of use	Final period
Reference group			
Red blood cells, $10^{12}/l$	8.25±0.04	8.27±0.06	8.27±0.07
Leukocytes, $10^9/l$	7.01±0.04	7.71±0.01	6.95±0.02
Hemoglobin, g/l	9.01±0.02	9.12±0.01	9.1±0.02
I experimental group			
Red blood cells, $10^{12}/l$	8.52±0.02	8.58±0.02	8.59±0.04
Leukocytes, $10^9/l$	6.77±0.01	6.21±0.03	6.42±0.02
Hemoglobin, g/l	10.0±0.04	11.22±0.04	11.51±0.01
II experimental group			
Red blood cells, $10^{12}/l$	9.21±0.02	9.75±0.03	9.85±0.02
Leukocytes, $10^9/l$	7.0±0.01	7.36±0.04	7.01±0.02
Hemoglobin, g/l	11.01±0.02	11.67±0.02	12.07±0.01
III experimental group			
Red blood cells, $10^{12}/l$	8.61±0.02	8.74±0.02	8.79±0.02
Leukocytes, $10^9/l$	7.30±0.01	7.35±0.01	7.10±0.03
Hemoglobin, g/l	10.02±0.03	10.63±0.03	11.12±0.01

Table 4: Characteristics of sperm of stud rams

Indicators	Ejaculate volume, ml	Mobility, score	Concentration, billion/ml	Resistibility, thousand
Preparatory period				
Reference	1.01±0.05	7.5±0.06	3.15±0.05	32.4±0.06
Group I	1.03±0.05	7.7±0.05	3.16±0.06	33.0±0.03
Group II	1.05±0.04	7.7±0.03	3.20±0.05	33.1±0.05
Group III	1.04±0.05	7.6±0.06	3.17±0.02	33.0±0.04
Period of use				
Reference	1.10±0.05	8.1±0.01	3.55±0.02	37.3±0.05
Group I	1.17±0.04	8.5±0.05	3.58±0.05	39.4±0.01
Group II	1.24±0.06	8.5±0.03	3.66±0.04	41.2±0.01
Group III	1.19±0.06	8.4±0.02	3.60±0.01	39.1±0.05
Final period				
Reference	1.18±0.03	8.5±0.04	3.55±0.06	40.5±0.02
Group I	1.30±0.04	9.0±0.06	3.54±0.04	42.2±0.05
Group II	1.37±0.05	9.2±0.02	3.65±0.05	43.5±0.06
Group III	1.32±0.02	9.0±0.05	3.60±0.03	42.8±0.04

Table 5: The results of influence of sperm quality on fertility of ewes and the number of offspring

Indicators	Group			
	Reference	Experimental I	Experimental II	Experimental III
	Heads (%)	Heads (%)	Heads (%)	Heads (%)
Inseminated ewes	45 (100)	42 (100)	40 (100)	41 (100)
Fertilized after first insemination	34 (75.5)	33 (78.5)	33 (82.5)	33 (80.5)
Lambled-out ewes	32 (71.1)	31 (73.8)	30 (75)	30 (73.2)
Obtained lambs	34 (106.2)	35 (113)	35 (116.7)	34 (113.3)

Thus, feeding stud rams with the apistimul preparation combined with the daily feed had a positive influence on the quantity and quality of sperm, and hence resulted in higher yield of offspring.

It should be noted that increasing the dosage from 15 to 20 mg/kg in the diet of rams did not result in any further increase in the indicators.

Based on the obtained results, one can conclude that the best option is adding the apistimul preparation to the main diet of rams in the dosage of 15 mg/kg.

Reference assessment of experimental stud rams' ejaculate quality was performed, and it has been found that they meet the requirements. Their ejaculates' volume ranged between 1.01 and 1.32 ml, the activity of spermatozoa in fresh ejaculates was 7.5-9.2 points, resistivity was between 32.4 and 43.5 thousand, and the fertilizing ability from the first insemination was between 78.5% and 82.5%.

Therefore, the materials obtained during the study provide a basis for potential use of the drone-brood homogenate and sodium chloride preparation in the conditions of disrupted spermatogenesis, or other conditions that result in sexual dysfunction of stud rams.

Apparently, adding into the diet of rams, the preparation with natural hormones – testosterone, progesterone, and estradiol – can stimulate the endocrine system and restore the function of testes and the general nervous system of animals, without disturbing the hormonal status of the animals.

Thus, the positive action of the apistimul preparation is expressed in the increased total weight and normal shape of spermatozoa that are inherent in a healthy animal, high quality of ejaculates from rams that meet the requirements of using sperm for insemination of ewes. The obtained results generally bespeak of expediency of using the drone-brood homogenate and sodium chloride preparation as a gonad protector for stud rams.

DISCUSSION

Larval jelly has narrow scope of application, but is unmatched in this scope. According to Baidan,^[32] Bonomi,^[11] and Bonvehi,^[33] the hormones contained in this product not only affect the organs of the endocrine system, but also, according to Lerker *et al.*,^[34] Lerker *et al.*,^[35] Rogala and Szymas,^[36] help restoring them. Our experiments showed that the apistimul preparation contributes to stabilization of the metabolism in stud rams and has a regulating effect on their central nervous system. Being a stimulant of the central mechanisms of regulating androgens formation, the preparation increases aqua-motor activity of the animals,

promotes rehabilitation of disturbed sexual function in rams, and increases the quality of ejaculates.

All these positive manifestations of growth, development, and quality of ejaculates in rams were due to the physico-chemical characteristics of drone-brood homogenate, the presence of several biologically active components, sex hormones, and sulfhydryl groups.

In several experimental studies, Goloshchapov,^[37] Krylov and Sokolsky,^[38] and Hismatullina^[39] observed the therapeutic efficiency of drone larvae for restoring the function of the actoprotector system and the sexual function in men. As a result of treatment, the motor function is activated, and sexual desire increases in men. The positive healing effect of royal jelly for treating oxidation and tumors has been noted by Echigo *et al.*,^[40] Li and Guan.^[41] During research on people, Matsui^[42] and Nakamura^[43] found that preparations based on beehive products were useful for protecting the organism from the impact of the environment and had immunomodulatory and actoprotective action. Our results confirm the influence of drone larvae on activation of the hematopoietic system and on stimulation of the central mechanisms that regulate generation of androgens, improve the motor and physical activity, and accelerate the regenerative processes in case of sexual dysfunction in animals.

The results of the experiments for studying the positive effects of the apistimul preparation on the ability to restore sexual dysfunction in rams, followed by obtaining high-quality ejaculates, are the basis for considering drone brood as an active adaptogen and a therapeutic product. Drone larvae are an effective, available, and inexpensive raw material for medicinal products, which is quite suitable for therapeutic treatment and prophylactics of valuable breeding males.

CONCLUSION

1. The developed drone-brood homogenate and sodium chloride preparation called apistimul contains a sufficiently large amount of biologically active components, including sex hormones such as estradiol (1004.36 ± 45.1 nmol/l), testosterone (4.56 ± 0.3 nmol/l), progesterone (81.22 ± 1.5 nmol/l), cortisol (331.4 ± 10.1 nmol/l), and prolactin (798.5 ± 7.3 nmol/l). It also contains the active part of many thiol-containing enzymes of sulfhydryl groups - $1098.6 \pm 20.3\%$. After vacuum freeze drying, the stabilized preparation is not inferior in its physico-chemical properties to the native homogenate drone brood.
2. Adding the apistimul preparation into the main diet in the dosage of 15 mg per 1 kg of live body weight has a positive effect on the overall condition of the animals, removes stressors, thus ensuring the adequate quality of sperm.
3. The experimental rams who received the preparation had

the tendency to increase the content of hemoglobin in the blood and physical activity. Adding the preparation in the dosages of 15 mg/kg once per day increased the volume of ejaculate to 30.4%, concentration of spermatozoa in the ejaculate up to 14.3%, mobility of spermatozoa increases to 9.2 points, and the fertilizing capacity of the spermatozoa after first insemination increases to 82.5%.

- The use of drone-brood homogenate has fully substantiated its efficiency as a source of medicinal and prophylactic product for removing long-term refusal to mate, ejaculation of poor-quality sperm, aggressiveness, passive behavior, and sluggish movement. After the use of homogenate, quality ejaculates inherent to healthy animals were obtained from rams, and the expected growth of resistivity and reproductive function in the animals was observed.

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Source of Support: Nil. **Conflict of Interest:** None declared.