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INFLUENCE OF HUMILID DRUG ON THE QUALITY OF SPERM PRODUCTION IN BREEDING BOARS UNDER DIFFERENT REGIMES OF USE

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Abstract

Background: Studies have shown the influence of the Red and White Belted meat breeding boars' usage regimes on the quality of sperm products under the effect of "Humilid" biologically active feed supplement. The data obtained in the control group of breeding boars are explained by their intensive use and significant consumption of body resources to maintain sexual activity. Due to the adaptogenic properties of the drug, which had a positive effect on the quality of sperm production in the experimental group of animals on the 30th day of the experiment, the body's resources were mobilized. It is established that consumption of biologically and ecologically safe feed supplements of humin nature permits to obtain high-quality and complete sperm products under different regimes of using breeding boars.

Methods: For the experiment, 10 animals of similar age and live weight were selected, 5 of which were the control group that did not receive the 'Humilid' drug and 5 animals that received a biologically active feed supplement with food. During the experiment, different regimes of boar usage were applied (once, twice and three times a week) for 60 days. The experiment was divided into three stages (preparatory, main, and final), during which sperm quality indices were monitored: ejaculate volume, sperm concentration, total number, and number of live sperm, their motility, and survival during incubation.

Results: In the previous period, the following dynamics in the effect of the effect of the regimens on sperm quality was observed: the twofold regime showed a decrease in ejaculate volume in the control group by 18.7 % and in the experimental group by 7.2 % compared to a single semen collection regimen. In the main period, the decrease in ejaculate volume was 17.6 % ($p<0.001$) and 5.4 % ($p<0.001$), respectively, and at the final stage – by 15.8 % ($p<0.001$) and 9.1 % ($p<0.001$). At that time, sperm motility and survival rates were the highest in animals that received "Humilid" biologically active feed supplement and had a sexual load twice a week. Sperm motility in the preparatory period in the control group was 90.4 % and in the experimental group – 95.0 %, survival – 84.0 % and 90.8 %, respectively. During the main phase of the experiment, sperm motility was within the range of 85.9 % and 98.8 % and survival – 78.5 % and 95.3 %, respectively. In contrast, in the final stage, sperm motility was 89.6 % and 92.0 % and survival was 82.0 % and 88.9 %, respectively.

Keywords: breeding boars, regimes of use, semen, sperm production, humin compounds.

INTRODUCTION

It is important that ejaculates obtained from breeding boars maintain a high level of quality throughout the period of use [1, 2]. Therefore, the physiological parameters of the breeding boar ejaculates are taken into account at the sampling points of [3]. Numerous studies have shown that the quality and volume of boar ejaculate depends on the breed [4, 5], age [6-8], testicle size [9-11], season [12-15] and seasonal duration of daylight. [16], microclimatic conditions and feeding [17] under intensive use or vice versa with an interval of semen collection every 2 months [5, 8, 18].

Sexual exercise regimens affect the quality and volume of sperm production and thus affect

the number of sperm doses and spermatozoa activity [19]. The authors [8, 18] also report similar results, according to which the highest volume of ejaculate is obtained with the usage regime of once a week. [20] According to the researchers, using different semen collection regimes after two, three, four, and seven days in terms of sperm motility in the period of once every seven days ($P < 0.05$) shows a result of 0.6, 1.9, and 3.3, % compared to 4, 3 and 2 days, respectively. But it shows that an interval of 7 days gives the best result.

At the same time, the data of the heat-resistance test show that the regime of sperm production is by seven times higher ($P<0.05$) – 4.9 % and 7.7 % compared to the 4 and 2 days regimes of use, respectively. In the experiments of the researchers, a study of oxidative processes was carried out to establish the level of thiobarbiturate compounds, where they were higher than in the two-day use regime ($P<0.05$) by 6.2 %, with the four-day interval by 11.1 % and with the seven-day interval by 9.1 %, respectively. Sperm integrity in these studies was compared to the results of the sperm collection regimen once a week ($P<0.05$) and was by 6.1 %, 7.5 % and 12.4 % higher compared to the groups of sperm collection regimens 4, 3 and 2 times a week.

Furthermore, studies by other researchers have shown that in large white boars, ejaculates collected at four-day intervals are characterized by larger volumes, higher numbers, and higher sperm motility than ejaculates obtained at one-day intervals [21]. According to investigators [18], the ejaculate of boars collected once or twice a week has the largest volumes, while those that were received daily have much smaller volumes. Researchers have found that the frequency of ejaculate selection affects the morphometric parameters of sperm [22-24], and this in its turn affects their fertilizing potential [25-27]. The influence of high frequency of ejaculate collection on physiological characteristics was also observed by foreign researchers [28]. Studies indicate a decrease in ejaculate volume and sperm concentration in Pietren boars, which were used twice a week compared to a one-day interval between successive ejaculate selections. The frequency also affected sperm motility, and was 20 % in intensively exploited boars' ejaculates, whereas the percentage with extensive use exceeded 80 %.

Sperm motility in ejaculate is considered to be one of the most important quality parameters that affects the fertilization potential [29]. The researchers found that the percentage of motile sperm ranged from 75.0 % with an ejaculate collection interval of five days, and up to

67.8 % with an interval of one day. Bonet et al. found that prolonged and frequent collection of ejaculate (every two days for 12 months) leads to a decrease in the percentage of spermatozoa with translational movement in the ejaculate. Similar conclusions were obtained by Strzeżek et al. [30], they found that the daily selection of ejaculate in boars for 10 days leads to a decrease in sperm motility to 54-58 %.

For industrial significance, one of the important indices is the number of live spermatozoa in the ejaculate, which have a rectilinear translational motion, because it shows how many sperm doses can be obtained from the ejaculate [31]. In the studies by Miclea et al. [32] ejaculate sampling with a seven-day interval provides 23 insemination doses per ejaculate, whereas ejaculates selected every 3 days provide only 18 insemination doses.

In addition to the boars' usage regimes, the quality of sperm production is influenced by the feeding factor in which much attention is paid to the addition of feed supplements to the animals' diet, because it is through the digestive system that the greatest impact on the boars' reproductive function is produced. One of such biostimulants is a humin compound. Humic acids are natural decomposed organic compounds of soil and brown coal, which are complex mixtures of polyaromatic and heterocyclic compounds with several side chains of carboxylic acid [33].

The use of humic acids in animal feed has a very short history. Researchers [34-35] first used humic acid to improve the immune system of calves. Whereas researchers [36] used humic acid to treat indigestion and diarrhea in cats and dogs respectively. Humic acid has also been used as an analgesic and antimicrobial agent in veterinary practice in Europe [37]. Humic acid has been found to have antiviral properties against herpes simplex and human immunodeficiency viruses [38, 39]. Many experimental studies have shown that humic acids are non-toxic and non-teratogenic [37]. Their use on the one hand can compensate for the deficiency of trace elements, and on the other – to carry out the typical for humic compounds effect on metabolic processes in animals, namely to stimulate them. In the liver, that is the organ in which the primary metabolism of humic substances is performed, the level of cyclic adenosine monophosphoric acid (c-AMP) raises, which indicates the presence of hormone-like action in them [40]. Thus, the above studies on the effects of humic compounds have been completely elucidated, but the effect of humates on sperm quality, under the different regimens of using meat Red and White-Belted boars, has been insufficiently studied.

MATERIALS AND METHODS

The studies were carried out at the Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences, Poltava, Ukraine. For the experiment 10 breeding boars of Red-White-Belted (RWB) meat breed were selected for each regime of use, five of which being the control – group I, and five being the experimental – group II, which were fed with standard feed containing “Humilid” biologically active feed supplement. Animals were selected by the method of analogues by age (18-24 months), live weight and quality of sperm.

Breeding boars were fed in compliance with the norms of the Institute of Pig Breeding and Agricultural Production of the National Academy of Agrarian Sciences, the animals had free access to feed and water. A biological supplement of humic nature was added to the feed mixture – the active substance in the amount of 1 % per liter of distilled water. The studies were carried out by the method of group-periods. The duration of the experiment was 60 days, in particular: period 1 – preparatory (sampling of initial samples), period 2 – the main (30 days) and period 3 – the final 930 days).

The regime of sexual activity was – collection of ejaculate once, twice and three times a week. The animals were kept individually. Boar semen was obtained by the manual method. The quality of sperm production was assessed according to ejaculate weight, concentration (refractometric method) and sperm motility (microscopy), as well as their survival during the three-hour incubation at the temperature of 38°C. The obtained digital material was statistically processed using the STATISTICA v. 10 software. Student’s t-test was used to compare the studied parameters and the intergroup differences, and the result was considered probable at $p \leq 0.05$.

RESULTS AND DISCUSSION

The data of the experiment in animals of the domestic Red and White Belted meat breed, indicate a significant impact of the breeding boars usage regime on the quality of sperm production, which coincides with the data of other researchers’ studies [18]. Thus, the regime of semen collection once a week contributes to an increase in ejaculate volume, concentration and total spermatozoa count [1, 2].

In our experiments, there was a decrease in the survival and motility of spermatozoa with intensive and extensive use of breeding boars in comparison with the optimum two-fold regime. It was already observed at the initial stage of the experiment (Table 1). The influence of high frequency ejaculate collection on physiological characteristics was also noted by other researchers [20] and its deterioration was noted. Semen collection from breeding boars twice a week reduced the volume of ejaculate at the initial stage of the experiment in the control group of breeding boars by 18.7 % and in the experimental group by 7.2 %, respectively.

Table 1. Impact of the regime of breeding RWB boars usage on the quality of sperm production depending on the sexual load in the preparatory period

Regime of use per a week, times	Group	Volume of ejaculate, ml	Sperm concentration, mln/cm^3	Total number of spermatozoa, bln	Number of spermatozoa in ejaculate, bln	Sperm motility, %	Survival of spermatozoa, %
1	I	250.9+5.11	0.232+0.02	58.20+1.44	49.47+2.08	85.0+2.39	78.7+2.31
II	237.0+6.78	0.215+0.07	50.95+3.98	43.81+1.67	86.7+3.01	82.0+1.78	
2	I	203.8+5.22	0.172+0.02	35.06+1.79	31.72+1.98	90.4+1.59	84.0+1.59
II	220.0+9.08	0.196+0.05	43.12+2.34	40.96+2.05	95.0+2.88	90.8+2.45	
3	I	161.5+6.49	0.179+0.04	28.13+4.51	23.09+1.06	82.1+2.94	63.8+2.07
II	155.6+7.11	0.167+0.02	26.08+2.05	21.46+2.87	82.3+2.75	70.9+1.94	

In the main period (the 30th day) the volume of ejaculate in boars of the control group decreased by 17.6 % ($p<0.001$) and in the experimental group – 5.4 % ($p<0.001$) compared to the preparatory period, the same trend was observed at the final experimental – stage 15.8 % ($p<0.001$) and 9.05 % ($p<0.001$), respectively. These data are consistent with the results obtained by researchers [18], who received a single ejaculate volume of 256 ml in a single regime of semen collection, 258 ml in a double regime, and 220 ml in a three-time regime. A similar trend was observed in terms of spermatozoa concentration and their total amount. The data were consistent with the results [18] and had deviations in the indices of concentration at a single semen collection regime of 289 * 10^6/ml and our obtained indices of 250 * 10^6/ml. Slightly different values were in the two-fold use regime – 276 * 10^6/ml and 203

* 10^6/ml, respectively, and in the three-fold regime – 240 * 10^6/ml and 161 * 10^6/ml.

Sperm motility rates under the double regime in the preparatory period were lower by 6.3 % in the control group and in the experimental group – by 9.6 %, respectively, at the main stage the indices decreased only in the experimental group of animals – 11.6 % ($p<0.001$), and in the final period – by 7.9 % in the control group and 5.9 % in the experimental one, respectively. The single semen collection regime also had a negative effect on sperm survival in both study groups and throughout the experiment. The results obtained by other researchers [18] indicate lower rates for once a week – 78.0 %, twice a week – 77.6 %, three times a week- 75.6 %, and daily collection – 70.1 %.

Also, during the monitoring of information on the experimental results, similar results of the Polish Landrace breeding boars' usage regimes were found [22]. The highest indices of sperm quality are established for a double regime of use and the corresponding data for a single per week regime with the rest interval of seven days per week.

With the twice a week (optimum) regime of boars usage, the quality of sperm production was the highest in terms of motility and survival of spermatozoa, it were those indices that most affect the percentage of fertilized ova. In the previous period, the mobility rate was 84.0 % in the control group and 90.8 % in the experimental group. During the main period, the index tended to decrease in motility compared to the previous period – 78.5 % ($p<0.01$) in the control group and significantly higher in the experimental group – 95.3 % ($p<0.001$), respectively. At the final stage of the experiment, the index was 82.0 % and 88.9 % ($p<0.001$).

Sperm survival in the twice a week regime increased compared to the regimes of single and thrice a week semen collection, this figure was higher at the beginning of the experiment and amounted to 84.0 % and 90.8 %, respectively. With the experiment continuation, the survival of spermatozoa in animals during the main period in the control group decreased by 6.5 % ($p<0.001$), and in the experimental group increased by 4.7 % ($p<0.001$) compared to the previous period. At the final stage, spermatozoa survival decreased by 2.0 % and 1.1 % ($p<0.001$) less than in the previous period, respectively (Table 2).

Table 2. Impact of the regime of breeding RWB boars usage on quality of sperm production

depending on sexual load in the main period

Regime of use per a week, times	Groups	Volume of ejaculate, ml	Sperm concentration, mln/cm^3	Total number of spermatozoa, bln.	Number of live spermatozoa in ejaculate, bln	Sperm motility, %	Survival of spermatozoa, %
1	I	237.5+2.67^3	0.243+0.05	57.59+2.65	50.33+2.98	87.4+2.56^3	75.3+1.87
II	257.3+3.15^3	0.250+0.06	64.25+3.02^3	56.54+3.76^3	88.5+1.03^3	85.6+1.55	
2	I	195.6+1.99	0.180+0.04	35.15+1.23	29.83+1.90^3	85.9+1.96^2	78.5+2.09^3
II	243.3+2.06^1	0.192+0.04	46.65+2.76^3	45.47+2.06^3	98.8+0.34^3	95.3+1.79^3	
3	I	172.1+1.07	0.140+0.03^2	24.08+2.59	19.48+1.78^3	80.9+1.78	70.9+1.11^3
II	186.0+1.27^3	0.171+0.02	29.41+1.08^3	24.41+1.88^1	83.0+1.56	80.0+1.86^3	

^{1}-p <0.05; ^{2}-p <0,01; ^{3}-p <0.001-. compared to twice a week optimum regime of breeding boars use.

With thrice a week usage regime, the quality of sperm production deteriorated with each stage of the experiment, due to the unnatural load on the animals and the depletion of their bodies. Permanent use of boars with such a regime can significantly worsen sperm production and lead to the phenomenon of aspermia, increase the number of defective form spermatozoa, as proven by practical studies [41].

In the course of the experiment, ejaculate values tended to deteriorate due to depletion of the animal's body. During the main phase of the experiment, the volume of ejaculate continued to decrease compared to the optimum regime by 12.3 % with a single usage of boars and by 30.8 % ($p<0.001$) with thrice a week usage. Sperm concentration decreased by 28.6 % ($p<0.01$) and by 12.2 %, respectively. The total number of sperm decreased by 45.7 % and 58.6 % ($p<0.001$). At that time, the number of live spermatozoa decreased by 53.1 % ($p<0.001$) and 86.2 %, and sperm motility increased by 6.1 % and 19.0 % compared to the previous period. Sperm survival decreased by 10.7 % ($p<0.001$) and by 19.1 % ($p<0.001$), respectively. At the final period of the experiment, the deterioration of sperm production slightly slowed down during the regimes of single and triple boars' usage, although it was

going on compared to the twice a week regime. Such data were confirmed by the experiments of other researchers, whose experiment lasted 100 days with cyclic repetition of sperm collection regimes [28] (Table 3).

Table 3. . Impact of the regime of breeding RWB boars usage on the quality of sperm production depending on the sexual load in the final period

Regime of use per a week, times	Groups	Volume of ejaculate, ml	Sperm concentration, mln/cm^3	Total number of spermatozoa, bln.	Number of live spermatozoa in ejaculate, bln	Sperm motility, %	Survival of spermatozoa, %
1	I	243.6+4.98^3	0.240+0.03	58.32+2.11	48.40+1.11	83.0+3.88	72.1+2.81^3
	II	248.5+3.65^3	0.245+0.01	60.76+2.49^3	52.25+1.06^3	86.8+3.97	80.3+2.54
2	I	205.0+5.48	0.183+0.01	37.51+1.59^3	33.38+2.54^1	89.6+3.09	82.0+4.77
	II	226.0+4.99	0.185+0.03	41.81+3.01	38.46+2.12^3	92.0+2.57^3	88.9+2.12^3
3	I	165.7+6.71	0.143+0.04	23.59+3.21^2	19.22+1.05^3	81.5+2.69^1	68.7+2.95^3
	II	180.2+4.84^3	0.166+0.02	29.91+2.11^3	24.04+2.61^3	80.4+3.93	78.2+2.70^3

^{1}-p <0.05; ^{2}-p <0.01; ^{3}-p <0.001- compared to twice a week optimum regime of breeding boars use.

Addition of the “Humilid” biologically active feed supplement to the diet helped to neutralize the intensive load and generally improved the quality of sperm products at all stages of the experiment. This has been reported in experiments by other researchers, who noted an improvement in meat quality, increased redness, marbling and a decrease in fat thickness [42], and they also reported more redness (7.02 vs. 5.48 points) when pigs were fed humic compounds, which included a higher level of humic acids (54.6 %); with a lower level of humic acid (12.2%), no such improvement was observed. This suggests that humic acid may be associated with meat color saturation, however, the exact mechanism underlying this has not been fully clarified to date. Humic substances also contain a minimum amount of minerals, including iron, manganese and copper. Also, an increase of copper levels in the body could affect the color of the pork, which was primarily the cause of the meat

discoloration. In addition, it should be noted that the decrease in the fat layer thickness and the ocurrence of the meat's marble structure is due to the distribution of protein and lipids under the influence of humic compounds. Accelerated myoglobin synthesis and fat deposition can also improve the meat color [43].

The experiment of researchers with addition of humic compounds to the diet of animals leads to improved outside appearance of meat [44], sperm production [45] and blood parameters [44], where during the 8-week period in the experimental group of animals the difference in relative lymphocyte levels was higher when using humates compared to the control group ($p<0.05$). In our studies on domestic Red and White Belt meat animals with a single regime of use in the main period, namely after the animals consumed the drug for 30 days, the volume of ejaculate increased by 8.6 % compared to the control group of animals.

Spermatozoa concentration increased by 2.8 %, simultaneously with an increase in their total amount by 10. 4 % ($p<0.001$), and the number of live spermatozoa – by 10.9 % ($p<0.001$), respectively. The motility of boars' sperm increased slightly, but the survival rate was by 10.2 % ($p <0.001$) higher compared to the control group of animals. In the twice a week animals usage regime with their receiving a biologically active feed supplement, there were positive changes in ejaculate indices, which is clearly seen in the tables. Twice a week use of boars in the experimental group showed an increase in the ejaculate volume by 19.6 % ($p<0.05$) compared to the control group of animals that did not receive a biologically active feed supplement. Sperm concentration increased by 6.3 %, while the total number – by 24.6 % ($p <0.001$), the number of live sperm in the ejaculate by 34.4% ($p <0.001$), with an increase in their motility – by 13.1 % ($p<0.001$), respectively. Sperm survival improved by 17.6 % ($p<0.05$), respectively. With thrice a week usage regime, the animals were depleted, and addition of a biologically active supplement to the feed helped to improve sperm production compared to animals that did not receive the drug of humic nature. Thus, the animals had a higher ejaculate volume by 7.5 % ($p<0.001$). Sperm concentration was higher, too – by 22.1 %, respectively, the total number – by 18.1 % ($p<0.001$), the number of live spermatozoa in the ejaculate increased by 20 %, and survival – by 12.8 % ($p<0.001$), respectively. On the 60th day of the experiment after the use of "Humilid" biologically active feed supplement, with a single use of boars in terms of the ejaculate volume, concentration, total number of spermatozoa and their live forms had a slight increase, whereas the sperm motility increased by 3.8 % . However, the sperm survival rate increased by 10.2% compared to the

experimental group. With twice a week use of boars in the experimental group, the ejaculate volume increased by 10.3 %, respectively.

The concentration increased insignificantly, but the total number of spermatozoa grew by 10.3 % ($p<0.001$). Also, the number of live spermatozoa increased in the ejaculate by 15.2 % ($p<0.001$) and reliably insignificant increase in the percentage of motile spermatozoa. Sperm survival improved by 7.8 % ($p <0.001$). It should be noted the natural decrease in sperm concentration in double and triple use regimes, due to an increase in the ejaculate volume and total sperm count. With intensive use of breeding boars, depletion of animals in the control group was observed. At the same time, the animals of the experimental group had a stabilization of the ejaculate quality, which was evidenced by other researchers [29]. Studies by other researchers [46], where experimental data showed a significant correlation between ejaculate parameters and the interval between ejaculate collection. Decrease in the time interval between successive ejaculate collections was accompanied by a decrease in its volume. The largest volumes were in the ejaculates, which were collected at seven-day intervals.

The increasing frequency of sperm collection, from the seven-day to the four-day interval, was accompanied by a gradual decline in the index. Ejaculate volumes collected every four, three and two days remained at the same level and were at least by 92 ml smaller than ejaculate volumes collected every seven days ($P\leq 0.05$). The time elapsed between successive ejaculate collections also affected the concentration and percentage of spermatozoa with translational movement. The concentration of sperm in ejaculates collected at seven-, six-, five-, four- and three-day intervals remained at a relatively high level [41].

CONCLUSIONS

1. The use of double sexual load on boars of both groups showed high quality sperm production in terms of motility in the main period of the experiment: 78.5 % in the control group of animals and 95.3 % in the experimental group, and survival amounting 85.9 % and 98.8 %.

2. The “Humilid” drug had the properties of an adaptogen and stimulated the body in

intensive and extensive regimes of use. Under the use of breeding boars twice a week in the experimental group, the ejaculate volume increased by 10.3 %, respectively. The concentration increased slightly, but the total number of sperm increased by 10.3 % ($p<0.001$). Furthermore, the number of live sperm in the ejaculate increased by 15.2 % ($p<0.001$) and there was a reliably insignificant increase in the percentage of motile sperm. Sperm survival improved by 7.8 % ($p<0.001$).

3. It should be noted that there was a natural decrease in the concentration of sperm in the two- and three-times a week regimes of use, due to the increase in ejaculate volume and the total number of sperm in the experimental group of animals. 'Humilid' is extremely effective in the irregular use of breeding boars – it allows them to be used as needed and obtain quality sperm production, without harming the body. The use of "Humilid" biologically active feed supplement is biologically safe for animals and the environment.

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