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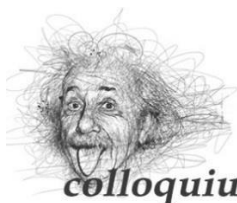
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QUALITY OF THE POLTAVA MEAT BREEDING BOARS' SPERM PRODUCTION DEPENDING ON THEIR USE REGIMENS AND UNDER THE EFFECT OF "HUMILID" FEED SUPPLEMENT

Abstract.

The influence of biologically active humic nature feed supplement on the quality of sperm production with different modes of using the Poltava meat breeding boars has been revealed. For the study, 10 animals of the similar age and live weight were selected, 5 of which being the control group that did not receive the "Humilid" drug and 5 animals that received a biologically active food supplement with feed. During the experiment, different modes of using boars (one, two and three times a week) have been used for 60 days.

The experiment was divided into the preliminary, main and final stages, during which the quality of sperm production was monitored: ejaculate mass, sperm concentration, total number and number of live spermium, their motility and survival during incubation outside the body. During the previous period of the experiment there was a clear dynamics of the impact of regimens on the sperm quality, namely the double regimen showed a decrease in ejaculate mass in the control group by 16.1 % and in the experimental group – by 5.7 % in the initial period compared to a single semen collection regimen.

In the main period, the decrease in ejaculate mass was 18.7 % ($p < 0.001$) and 12.5 % ($p < 0.001$), respectively, and at the final stage by – 16.2 % ($p < 0.001$) and 10.8 % ($p < 0.001$). At that time, sperm motility and survival rates were the highest in animals that received the biologically active feed supplement "Humilid" and had a sexual load 2 times a week. Sperm motility in the control group was 96.0 % and in the experimental group – 97.0 %, survival was 80.2 % and 83.8 %, respectively. During the main stage of the experiment, sperm motility was within the range of 90.4 % and 96.1 %, respectively. At the final stage, sperm motility indices were 90.6 % and 94.0 % and survival indices were 86.4 % and 91.8 %, respectively.

Such data in the control group of breeding boars are explained by the depletion of the body's resources with their intensive use. Due to the drug's adaptogenic properties, it had a positive effect on the quality of sperm production in the experimental group animals and accumulated body resources on the 60th day of the experiment. Thus, it was established that the consumption of biologically safe feed additive "Humilid" by animals will permit to obtain high-quality and complete sperm products under different regimens of using breeding boars.

Keywords: breeding boars, modes of use, semen, sperm production, humic substances.

INTRODUCTION

Violation of the boars use regimes leads to deterioration in the sperm quality. The percentage of motile sperm in ejaculates collected at seven- and six-day intervals between successive selections remains at a relatively high and similar level, approximately 72.8–73.9 %, and it even slightly increases (up to 75.0 %) at five days intervals. Increasing the frequency of ejaculation selection with an interval of less than five days leads to a decrease in the sperm motility in ejaculates. The index of actively motile sperm in ejaculates collected at one-

day intervals was 67.78 %, or by 7.2 % less than in ejaculates obtained at five-day intervals. This difference was significant at $P \leq 0.05$ (Bajena et al., 2016) [1]. The most important characteristic of ejaculate fertilization is the total number of motile sperm. It determines the number of insemination doses that can be prepared of a single ejaculate (Kondracki et al., 2013) [9]. The data show that with an interval of seven, six or five days between consecutive collections, the number of sperm in the ejaculate remains at a very high level (more than

90 billion motile sperm). Reducing the intervals between consecutive collections to four, three, two and one days leads to a decrease in the number of sperm in the ejaculate by at least 10 billion per day. Similar trends were observed in the case of the number of insemination doses obtained from one ejaculate. The data show that ejaculates obtained at intervals of seven and six days provided the highest number of insemination doses (approximately 38 doses per ejaculate). In turn, the reduction of time between ejaculate collection led to a decrease in the number of obtained insemination doses. Ejaculates collected at intervals of three, two and one day provided 12, 19 and 28 insemination doses, respectively, less than ejaculates collected at intervals of six or seven days ($P \leq 0.05$) (Bajena et al., 2016) [1]. Frequent semen collection is accompanied by a decrease in the concentration, motility, survival and resistance of sperm, a decrease in the ejaculate volume, fertility. With intensive use regime (once a day and every other day), the sexual activity of boars is high at first, and then (after 2–3 months) sharply decreases and can lead to aspermia and depletion of the whole body (Fülöp, 1996) [5]. The sperm quality deteriorated during prolonged sexual rest of boars (Kádár et al., 2005) [7]. With extensive use (1 time in 6–10 days) the number of motile sperm and their survival is significantly reduced (Miclea et al., 2007) [11]. Damaged and defective spermatozoa appear in ejaculates (Frangéž et al., 2005) [4].

The load in breeding boars in natural mating use twice a week only makes 50–70 sows, and with artificial insemination – 300–500 heads per year (Shvetsova, 2014) [15]. Sexual activity and sperm quality of boars depends not only on the quality of feed, but also on the mode of boars' use, housing conditions and breed characteristics (Kemp et al., 1991) [8]. In the commercial use of valuable breeds certified boars, it is important to obtain high-quality and complete ejaculate in the maximum amount. One of the efficient factors in improving sperm production is the effect of feed additives on the body through the digestive system of animals, as the reproductive system of boars responds to changes in nutrition very quickly (Kondracki et al., 2018) [13]. The mechanism of humic substances' action on the permeability of the cell membrane is explained by the slowing down absorption of organic compounds. However, they enhance the transport of inorganic cations due to their active inclusion in the chelation reaction (Ji, 2006) [6]. The property of humates to form chelated compounds with metals, in particular with trace elements, permits to increase the minerals availability in animals. Their use on the one hand can compensate for the deficiency of trace elements, and on the other hand – they produce the typical for humic compounds effect on metabolic processes in animals, namely to stimulate them (Shvetsova, 2014) [15]. In the liver, which is the organ where the primary metabolism of humic substances occurs, the c-AMP level of increases, which indicates their hormone-like action (Ji, 2006) [6]. The use of sodium humate in diets not only stimulates the course of erythropoietic processes in the animal body, but also provides a metabolic adjustment aimed at improving the efficiency of oxidative tissue processes. It also raises weight gain and resistance, i.e. the number of lymphocytes that play an important and integral role in protecting the body. They stimulate the development of immunity. Therefore, the increase in the relative number of lymphocytes observed in studies has shown that

humic compounds can have a beneficial effect on the animal's immune system. The humic substances' mechanism of action is related to their ability to form complex compounds in the body that function as modulators of intercellular interaction. They maintain the balance of activity in the immune system. Humic acid stimulates the body's resistance, which stimulates an increase in phagocytic activity. (Zerk et al., 1991) [18]. It has also been previously suggested that humic acid could stimulate the production of glycoproteins that could regulate the immune system by maintaining the balance of killer and T cells. However, it should also be noted that the systematic consumption of humic acids has previously been associated with human diseases, therefore, the safe use of humic compounds is required (Wang et al., 2008) [17]. Experience with the use of humates is quite common in Europe and Ukraine, in poultry farming, crop husbandry and stock-raising, namely in pig breeding (Moskalenko, 2018) [12], but it is understudied as to the impact on sperm quality depending on various factors. Our experiments were performed to study the effect of humic compounds on the quality of sperm production in breeding boars under different modes of sexual use.

MATERIALS AND METHODS

The studies were performed at the Institute of Pig Breeding and Agroindustrial Production of NAAS, Poltava, Ukraine. For the experiment, 10 Poltava meat (PM) breeding boars were selected for each mode of use, five of which were the control – group I, and five experimental ones, which were fed biologically active feed additive “Humilid” with standard feed – group II, including animals similar by age (18–24 months), live weight and quality of sperm. Breeding boars were fed in compliance with the norms of Institute of Pig Breeding and Agro-Industrial Production of NAAS, the animals had free access to food and water. A biological supplement was added to the feed – a humic nature substance which had the concentration in the amount of 1 % per liter of distilled water. The study was performed by the method of groups-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 30 days. The sexual load mode was –collecting 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 by ejaculate weight, concentration (using the refractometric method) and sperm motility (microscopy), as well as their survival during a 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 inter-group differences, and the result was considered reliable at $p \leq 0.05$.

RESULTS AND DISCUSSION

The experimental data indicate a significant impact of the boars use regime on the quality of sperm, which was studied in other works of domestic (Shvetsova, 2014, Shostia et al., 2018) [15, 16] and foreign researchers (Bajena et al., 2016, Frangéž et al., 2005, Kondracki et al., 2013) [1, 4, 9]. Also the positive effect of humic nature feed supplements on other animals and birds was studied (Moskalenko, 2018) [12]. The single weekly sperm collection regime promotes

the increase of the ejaculate mass, concentration and the total sperm number (table 1).

Table 1.

Impact of the of breeding boars use regime on the quality of sperm depending on sexual activity in the previous period

Regime of use for a week, frequency	Group	Ejaculate mass, ml	Sperm concentration, billion.	Total number of spermatozoa, billion	Number of live spermatozoa in ejaculate, billion	Sperm motility, %	Sperm survival, %
1	I	255.56±5.56	0.24±0.01	61.33±1.55	51.17±2.47	83.40±2.27	72.50±2.16
	II	263.45±7.64	0.26±0.04	68.38±2.49	58.53±1.96	85.60±1.23	74.00±1.06
2	I	220.05±9.13	0.19±0.02	42.90±2.21	41.21±2.35	96.00±0.13	80.20±1.14
	II	248.50±6.11	0.24±0.01	60.76±1.48	58.94±2.98	97.00±0.08	83.80±1.07
3	I	197.15±9.22	0.19±0.02	38.83±2.21	30.60±1.52	80.00±1.54	65.60±2.50
	II	205.50±5.11	0.20±0.04	41.10±2.70	33.71±1.94	82.40±1.36	69.40±2.87

At the same time, there is a decrease in survival and mobility compared to the optimal twofold regime. Collection of sperm from breeding boars twice a week reduces the mass of ejaculate in animals at the end of the previous period in the control group by 16.1 % and in the experimental group – by 5.7%. At the end of the main period (table 2) the indices were: in the control PM boars – 18.7 % ($p < 0.001$) and in the experimental group – 12.5 %.

A similar trend was observed at the final stage of the experiment – 16.2 % ($p < 0.001$) and 10.8 % ($p < 0.001$), respectively. These data are consistent with the results obtained by researchers (Frangéž et al., 2005) [4], the volume of sperm obtained with a single collection regime was 256 ml, with a two-fold regime – 258 ml, with a three times collection regime – 220 ml. There was a similar trend in the indices of sperm concentration and total number, the data were consistent with the results (Frangéž et al., 2005) [4] and there were slight deviations in the concentration at a single semen collection of $289 \times 10^6/\text{ml}$ and in our obtained indices – $260 \times 10^6/\text{ml}$.

Slightly different values were in the twofold regime of use $276 \times 10^6/\text{ml}$ and $240 \times 10^6/\text{ml}$, respectively, and in the three-times regime $240 \times 10^6/\text{ml}$ and $220 \times$

$10^6/\text{ml}$. The total number of spermatozoa differed significantly according to the researcher (Frangéž et al., 2005) [4], only when three times regime was used 51, the value was 51×10^9 , compared to the results of our experiment – 41.1×10^9 . Whereas the sperm motility index was consistently lower and amounted to 15.1 % in the control group in the previous period and 13.3 % – in the experimental period, during the main period the control group had a decrease in the index of 6.4 % ($p < 0.001$) and 10.4 %, respectively, and in the final period – 11.4 % and 11.6 % ($p < 0.001$), respectively.

The results obtained by other scientists (Frangéž et al., 2005) [4] indicate lower rates at the regimes of once a week – 78.0 %, twice a week – 77.6 %, three times a week – 75.6 %, and at daily collection – 70.1

%. Also, when monitoring the information on the results of experiments, similar results of the influence of Polish Landrace breeding boars' use regimes (Kon-dracki et al., 2018) were found [13]. The highest indicators of sperm quality were established there, with a twofold regime of use and the appropriate data with a single regime of use per week with a rest interval of seven days a week.

Table 2.

Influence of the PM breeding boars' regime of use on the quality of sperm production depending on the sexual load in the main period

Regime of use for a week, frequency	Group	Ejaculate mass, ml	Sperm concentration, billion.	Total number of spermatozoa, billion	Number of live spermatozoa in ejaculate, billion	Sperm motility, %	Sperm survival, %
1	I	250.90±5.11 ³	0.23±0.02	58.20±1.44 ³	49.52±3.64	85.00±2.39 ³	75.70±2.31 ³
	II	256.50±4.03	0.25±0.03	64.25±3.24 ³	55.92±2.47 ³	87.00±1.96	82.30±1.87
2	I	203.80±10.22 ³	0.17±0.02	35.06±1.79 ³	31.72±2.04 ³	90.40±1.59 ³	82.00±3.61 ²
	II	224.30±7.25 ³	0.19±0.04	42.33±2.04 ³	40.68±0.14 ³	96.10±2.56	89.80±1.23 ¹
3	I	181.50±10.49 ³	0.17±0.02	31.95±2.13	26.25±1.96 ³	82.10±2.95 ¹	63.90±2.41 ³
	II	199.30±6.18 ³	0.19±0.04	38.20±1.87	33.01±3.01	86.40±3.59 ²	78.10±4.63 ³

¹- $p < 0.05$; ²- $p < 0.01$; ³- $p < 0.001$ - compared to twofold standard regime of breeding boars use.

In our studies, a single semen collection regimen negatively affected the sperm survival in both study groups throughout the experiment. In breeding boars this index was the lowest in the previous period with intensive use three times a week – 21.9 % in the control group and 19.5 % in the experimental group; in the

main period – 28.3 % ($p < 0.001$) and 14.9 % ($p < 0.001$), respectively; and in the final period – 27.1 % ($p < 0.001$) and 14.7 % ($p < 0.001$), respectively, compared to the twofold regime, the survival in which was the highest among other regimes (table 3).

Table 3.

Influence of the PM breeding boars' regime of use on the quality of sperm production depending on the sexual load in the final period

Regime of use for a week, frequency	Group	Ejaculate mass, ml	Sperm concentration, billion.	Total number of spermatozoa, billion	Number of live spermatozoa in ejaculate, billion	Sperm motility, %	Sperm survival, %
1	I	240.3±8.36 ³	0.25±0.01	60.24±2.37	48.79±2.30 ³	81.30±1.22	78.10±2.82
	II	251.4±5.47 ³	0.25±0.03	63.25±1.68 ³	53.13±1.93 ³	84.20±2.19 ³	80.50±2.47
2	I	201.3±6.79 ³	0.20±0.01	40.20±3.78	36.18±1.39 ³	90.60±0.75 ¹	86.40±1.15 ³
	II	224.1±5.60 ³	0.19±0.01	42.78±2.06 ³	40.21±3.16 ³	94.00±0.55 ³	91.80±1.43
3	I	178.9±7.65 ³	0.18±0.04	32.93±2.04 ³	27.99±2.00 ³	85.20±1.33 ³	68.10±1.11 ³
	II	192.6±5.87 ³	0.17±0.03	34.17±1.58 ³	30.07±1.54 ³	88.30±2.19 ¹	82.10±1.46 ³

¹- $p < 0.05$; ³- $p < 0.001$ - compared to twofold standard regime of breeding boars use.

With the twofold optimal regime of breeding boars use, the quality of sperm production was the highest in terms of motility and survival, namely in those indices that effect the most on the percentage of the ovum fertilization. In the boars of the control group in the previous period, the mobility rate was 96.0 % and in the experimental group – 97.0 %. During the main period, the index above tended to decrease compared to the previous period by 90.4 % ($p < 0.001$) and 96.1 %.

At the final stage of the experiment, the indices were 90.6 % ($p < 0.05$) and 94.0 % ($p < 0.001$), respectively. Survival with the twofold regime increased compared to the regimes of single and triple semen collection per week, this figure was already higher at the beginning of the experiment and amounted to 80.2 % and 83.8 %. During the experiment, sperm survival in the control group in the main and final periods tended to increase slightly in the main period – by 2.5 % ($p < 0.01$) and 8.2 % of the experimental, and in the final period – by 8.0% ($p < 0.001$) and 10.6 % compared to the initial period.

Also, when monitoring the information on the experiment results, the similar data were found on the effect of the regimes of Polish Landrace breeding boars use (Kondracki et al., 2018) [13] where were the highest indices of sperm quality with the twofold use regime and the respective data on the single use regime per a week with the rest interval of seven days.

With thrice a week using 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. Constant use of breeding boars with this regime can lead to a significant deterioration of sperm production and lead to the onset of

aspermia and an increase in defective forms of spermatozoa, as proven by practical studies (Bassols et al., 2005) [2].

Thus, the mass of ejaculate in the previous period decreased by 10.4 % and 20.9 % compared to the twofold use regime. The total number and quantity of live spermatozoa and their motility also decreased significantly. The number of live spermatozoa was by 3.3 % and 16.0 % higher, respectively.

The results tended to deteriorate as the experiment continued, due to depletion of the animals' bodies. During the main stage of the experiment, the mass of ejaculate continued to decrease compared to the optimal regime by 12.8 % ($p < 0.001$) with a single use of boars and by 12.5 % ($p < 0.001$) with thrice a week use, respectively. Sperm concentration decreased insignificantly. However, the total number of sperm decreased by 9.7 % and 10.8 %, respectively.

At that time, the number of live spermatozoa decreased by 20.8 % and 23.2 %, respectively. Sperm motility increased with a single use regime compared to the previous period, but was slightly less than with a double use regime – by 10.1% and 11.2 ($p < 0.01$), respectively, compared to the initial results. Sperm survival decreased by 2.6 % ($p < 0.001$) during the main stage and increased by 3.6 % ($p < 0.001$) during the final stage.

At the final period of the experiment, the deterioration of sperm production in the regimes of single and triple use of boars per week slowed down somewhat, although it continued to deteriorate in comparison with the twofold use regime. Such data were confirmed by the experiments of other researchers, whose experiment

lasted 100 days with cyclic repetition of sperm collection regimes (Kondracki et al., 2013) [9].

It should be noted that adding the "Humilid" biologically active feed supplement to the diet helps to neutralize the intense load and generally improves the quality of sperm at all stages of the experiment. It has also been recorded in the experiments of other researchers, who have noted an improvement in the quality of meat, increased redness, its marbling and a decrease in fat thickness. Similarly (Ji et al., 2006) [6] greater redness was also reported (7.02 vs. 5.48 points) when pigs were fed humic compounds, which included higher levels of humic acids (54.6 %); with lower levels of humic acid (12.2 %), no such improvement was observed. This suggests that humic acid may be associated with meat color intensity, however, the exact mechanism underlying this fact has not been fully studied to date. Humic substances also contain a minimum amount of minerals, including iron, manganese and copper. Elevated Cu levels in the pig's body can also effect the color of pork, which can be another cause of meat coloration change. Besides, it should be noted that the decrease in the fat layer thickness and the occurrence of the marbled structure in the meat is due to the distribution of protein and lipids under the influence of humic compounds. Accelerated myoglobin synthesis and fat deposition can also improve meat color (Xu and Feng, 1998) [19]. The experiment of researchers with addition of humic compounds to the animals' diet leads to improvement in the appearance of meat (Wang et al., 2008) [17], sperm products (Shostia et al., 2018) [16] and blood parameters (Wang et al., 2008) [17]. During the 8-week period in the experimental group of animals, the difference in the relative level of lymphocytes was higher with the use of humates than that in the control group ($p < 0.05$).

In our studies, with the single use regime in boars of the experimental group in the main period, namely after the use of the "Humilid" drug for 30 days, the volume of ejaculate increased by 2.1 % compared to the control group of animals. Sperm concentration increased by 7.5 %, simultaneously with the increase in their total amount – by 9.4 % ($p < 0.001$) and the number of live sperm – by 11.4 % ($p < 0.001$). Sperm motility increased slightly, but their survival increased by 8.7 % compared to the control group of animals.

At the twofold optimal regime of using experimental group animals, receiving a biologically active feed supplement, had positive effects, which are clearly presented in the tables. Twofold use regime of boars in the experimental group showed an increase in ejaculate volume by 10.2 % ($p < 0.001$). Sperm concentration index increased insignificantly, while their total number increased by 6.0 %, the number of live sperm in the ejaculate – by 11.1 % ($p < 0.001$), with a slight increase in their motility. Sperm survival improved by 9.5 % ($p < 0.05$).

When used threefold, the animals were exhausted, and addition of a biologically active supplement to the feed helped to improve sperm production compared to the animals that did not receive the drug of humic nature. Thus, the animals at the main stage of the experiment had a higher ejaculate mass by 8.9 % ($p < 0.001$),

sperm concentration – by 8.3 %, total spermatozoa number – by 16.3 % and the number of live spermatozoa in the ejaculate – by 20 %, and survival – by 22.2 % ($p < 0.001$).

On the 60th day of the experiment after the use of animals of the experimental group in a single regime of use, the mass of ejaculate increased by 4.6 % ($p < 0.001$), the concentration was on a par with the control, the total number of spermatozoa – by 4.9 % ($p < 0.001$), the number of live spermatozoa in the ejaculate increased by 8.9 % ($p < 0.001$), motility – by 3.5 % ($p < 0.001$), survival – by 3.0 %.

Under the twofold use regime, ejaculate mass increased by 11.3 % ($p < 0.001$), the total number of spermatozoa – by 6.4 % ($p < 0.001$), the number of living – by 11.1 % ($p < 0.001$), motility increased by 3.4 % ($p < 0.001$), and survival – by 6.3 %.

With intensive use, the volume of ejaculate in the animals of the experimental group increased compared to the control group by 7.7 % ($p < 0.001$), the total number of spermatozoa – by 3.7 % ($p < 0.001$), the number of live spermatozoa in the ejaculate – by 7.4 % ($p < 0.001$), motility – by 3.6 % ($p < 0.05$), survival – by

16.2 % ($p < 0.001$). It should be noted that there was a natural decrease in the concentration of sperm with the twofold regime of use by 5.2 % and with the triple regime – by 5.9 %, due to an increase in the ejaculate mass and the total number of spermatozoa.

With the intensive use of breeding boars, depletion of animals in the control group was observed. Similar results have been found in scientific papers (Bajena et al., 2016) [1], where the data show a significant correlation between ejaculate parameters and the interval between ejaculate collection.

A decrease in the time interval between successive ejaculate collections was accompanied by a decrease in ejaculate volume. The largest volumes were in the ejaculates, which were collected with seven-day intervals. The increasing frequency of sperm collection, from a seven-day to a four-day interval, was accompanied by a gradual decline in the above index. Ejaculate volumes collected every four, three and two days remained at the similar 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 sperm concentration in the ejaculate and the percentage of spermatozoa with translational movement. Sperm concentrations in ejaculates collected at seven-, six-, five-, four-, and three-day intervals remained relatively high (Bassols et al., 2005) [2].

At that time, the experimental group animals had a stabilization of ejaculate quality, and generally had better performance, as evidenced by other researchers (Kondracki et al., 2018) [13].

Conclusions

The twofold regime of the sexual load on boars in both groups showed high quality sperm production in terms of motility – 96.0 % in the control group animals and that in the experimental group – 97.0 % and survival – 80.2 % and 83.8 %. The "Humilid" drug had an adaptogenic and stimulating effect on the body with in-

tensive and extensive regimes of use. "Humilid" is extremely effective with the unstable regime of boars use – it permits to use them as needed and to obtain quality sperm, without harm to the body. The use of biologically active "Humilid" feed supplement is biologically safe.

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