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RESEARCH ARTICLE

Welfare and sperm quality with a focus on stress resistance of *Bos taurus*

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We studied the effect on the welfare of bulls (n=16) of their resistance to stress. The bulls with less stress resistance (group II) were in a standing position during 24 hours 53.6 minutes longer than their highly resistant peers (group I). On the contrary, the bulls of the 1st group rested lying 87 minutes longer. The welfare of the bulls, due to different response to stress and behavioral patterns, affected sperm quality. Defect of sperm not suitable for use compared to peers of group I was higher in bulls of group II by 12.9–22.2% in the first three years of breeding.

Key words: Hormones and enzymes; Behavioral patterns; Functional indices; Sperm productivity

Introduction

The animal welfare science, although it is relatively new, has already a large complex of multidisciplinary literature in various scientific fields, ranging from more theoretical (for example, how to assess animal motivation and well-being) to more practical ones (for example, how to improve treatment with animals, reduce injuries). World Organization For Animal Health (OIE) has recently begun developing a global standard for animal welfare, linking product quality with it. Therefore, the goal of all such studies is ultimately to obtain new knowledge that will allow us to objectively evaluate and improve the well-being of animals in real conditions (Fraser, 2018). But if we do not take into account the body's resistance to stress, then in the end it can distort the results of any other assessment of animals (Davis, 1993). The welfare of bulls *(Bos taurus)*, under constant operating loads, largely depends on the strength of their skeletal system and the body as a whole. While it was established (Chrousos, 2000) that under stress glucocorticoids block the effects of growth hormone, thyrotropin, inhibit the activity of osteoblasts, which leads to deterioration of the condition of bones and the occurrence of osteoporosis of bones, a depressive state occurs, sexual function and the immune system are suppressed, and obesity can occur. The results of scientists' studies indicate that stress takes the body out of balance and often leads to impaired reproductive function, poor health, and decreased animal productivity (Grandin & Shivley, 2015; Chernenko at al., 2017; Kastelic et al., 2018; Kasimanickam et al., 2019). Under the conditions of industrial technology for keeping bulls, their body is constantly exposed to operational loads that can cause them to become stressed. Their transfer from one group to another always leads to a rank struggle (McVeigh et al., 1982). Stress occurs not only in a displaced animal, but in the entire group (Warriss et al., 1984). Changing the diet, getting used to certain modes of maintenance can also lead to disruption of homeostasis. Veterinary measures related to routine blood sampling from bulls, vaccination, insertion of a nasal ring, clearing hooves, forced exercise, and training them to excrete sperm from an artificial vagina often cause stress (Lockwood et al., 2016). In recent years, even for temperate regions, heat stress has become relevant (Mylostyvyi & Sejian, 2019). In the absence of need, earlier selection of bulls in many countries was not aimed at increasing the heat resistance of their body. Therefore, it is necessary to develop environmentally sound criteria for improving animal welfare (Carroll & Forsberg, 2007; Chenoweth & McPherson, 2016). Indeed, under heat stress, the well-being of animals can change as a result of the combined action of the nervous and endocrine systems (Knol, 2011; Luceño et al., 2019), as well as the clinical parameters of the body and blood composition (Brito et al., 2002). This is especially true in summer, when summer heat negatively affects the body without an additional cooling system (Soren et al., 2018). A significant effect of heat stress on sperm abnormalities, the integrity of the acrosome and plasma membrane of both fresh and thawed sperm of bulls has been established (Ahirwar et al., 2018). Therefore, during the heat season, it is recommended to

provide additional cooling of the rooms (large-diameter axial fans, drip irrigation, as well as their combination), since the temperature and humidity index inside animals can be 2–3 units higher than outside, which can negatively affect on their well-being (Mylostyvyi et al., 2019).
An increased understanding of the effects of stress on livestock has emerged throughout the scientific world. The purpose of this article is to show the relationship between the welfare of bulls and their stress resistance, which will probably allow them to be exploited more rationally.

Materials and Methods

The experimental part of the work was carried out in the conditions of the Dnepropetrovsk regional state enterprise on pedigree breeding in animal husbandry on 16 adult bull-sires of Holstein breeds. The studies were carried out in accordance with the state project "Theoretical substantiation and practical implementation of selection for improving the technological and productive qualities of farm animals and poultry", state registration number: 0110U007614 (2010–2020). The age of the breeding bulls was 5–5.5 years. Experimental animals were kept under a summer canopy in individual stalls with an area of 18 m^2. The average regime of sexual load on bulls per week is one doublet use. The daily feeding ration per 100 kg of live weight in nutrition was 12 MJ of exchange energy and 155 g of digestible protein. The serum cortisol concentration was determined on a *Stat Fax-2100* microplate immunoassay analyzer using the *Diagnostic system laboratory* reagent kit (USA). The determination of AST and ALT activity in blood serum was carried out with use a *1904Vet* semi-automatic biochemical analyzer using diagnostic reagent kit from *La Hema* (Czech Republic).
The reaction of bulls to stress was determined by examining the reactivity of the hypothalamus-pituitary-adrenal gland system to the stress load accompanying blood sampling: rigid simultaneous fixation of animals with a self-fixer for the head and additionally for the nasal ring with jugular vein squeezing, change in feeding regimen, due to taking blood, the presence of a veterinarian and strangers (support staff for taking blood). The concentration of the hormone cortisol in the blood of experimental animals was studied under stressful conditions and one hour after it according to the methodology (Hopster et al., 1999). According to the reaction to stress, bulls are divided into groups (Table 1), taking into account the range of the reference norm (Kondrahin, 2004). The ratio of the maximum value of the reference norm (110.40 nmol/L) to its minimum value (57.96 nmol/L) gives a result of 1.9, i.e., up to 2.0. We hypothesized that an increase in this ratio may indicate a stronger response of animals to stress. Pedigree bulls that had a ratio (CS_2/CS_1) <2.0 were distributed in group I (n=9) with greater resistance to stress. The animals with a ratio of (CS_2/CS_1) ≥2.0 into group II (n=7), with less resistance to stress.
A week after the study of stress resistance in bulls, daily ethological observations were carried out according to generally accepted methods. Behavior indicators of each bull were recorded during the day every five minutes, using the alphabet of the main elements of the bull's behavior: : L – lies, St – stands, M – moves, E – eats, Dr – drinks, G – gum, Sl – sleeps, U – urination, De – defecation, etc. All functional actions of bulls were counted into functional indices of animal behavior (Velikzhanin, 1995). Functional behavior indices were calculated using the formulas: $T_1=\Sigma t_1/t$, where T_1 is the index of food activity; Σt_1 is the amount of time spent on eating food and chewing gum, min.; – 1440 min., total observation time; $T_2=\Sigma t_2/t$, where T_2 is the index of physical activity (active state of the animal); Σt_2 is the amount of time spent on eating food and chewing gum, urination, defecation and body care, min.; $T_3=\Sigma t_3/t$, where T_3 is the inactivity index (passive state of the animal); Σt_3 is the amount of time spent on resting while standing or lying down, min.; $T_4=\Sigma t_4/t$, where T_4 is the index of comfort actions; Σt_4 is the amount of time spent on acts related to urination, defecation and body care, min.; $T_5=\Sigma t_5/t$, where T_5 is sleep index, %; Σt_5 is the amount of time spent on sleeping, min.
Sperm productivity indices of bulls were analyzed for the first three years of their exploitation using generally accepted methods for native sperm.
The experimental part of the studies was carried out in accordance with the requirements of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). Analysis of experimental data was performed using Statistica 10 (StatSoft, Inc., USA).

Results

The dynamics of the concentration of hormones and enzyme activity during the experiment in comparison with the reference norm are reflected within the data of Tables 1 and 2.

Table 1. Blood indicators of breeding bulls of Group I.

Indicators	Before stress load (control)	After a stressful load (experiment)	Reference norm
Cortisol, nmol/L	94.6 ± 22.1	147.8 ± 38.7*	57.96–110.4
Testosterone, nmol/L	16.4 ± 3.1	21.1 ± 5.4*	7.03–20.67
Creatine phosphate kinase, nmol/s•L	931.7 ± 274.9	1163.2 ± 352.2	333.0–1667.0
Alanine aminotransferase, nmol/s•L	185.4 ± 14.8	878.1 ± 172.2*	166.7–500.1
Aspartate aminotransferase, nmol/s•L	236.1 ± 49.8	900.2 ± 253.2*	166.7–833.5

Note: * – Significant difference (P<0.05) to the Mann-Whitney U-test.

The stress response in bulls of group I was not sharp, but pronounced. This follows from the fact that after a stress load, an increase in the concentration of cortisol by 1.3 times relative to the upper limit of the reference norm is observed in them, as well as ALT by 1.8 times and AST by 1.1 times. The concentration of testosterone reached the upper limit of the norm, and only the activity of creatine phosphate kinase remained within its limits. 1 hour after the stress load, cortisol reached a concentration exceeding the control by 53.2 nmol/L (P<0.05), and testosterone by 5.1 nmol/L (P<0.05), ALT activity increased by 692.7 (P<0.05) and AST by 664,1 nmol/s•L (P <0.05).

Table 2. Blood indicators of bulls of group II.

Indicator	Before stress load (control)	After a stressful load (experiment)	Reference norm
Cortisol, nmol/L	100.1 ± 17.6	387.3 ± 109.4*	57.96–110.4
Testosterone, nmol/L	17.6 ± 7.1	34.4 ± 7.7*	7.03–20.67
Creatine phosphate kinase, nmol/s•L	1078.8 ± 351.2	1728.9 ± 410.2*	333.0–1667.0
Alanine aminotransferase, nmol/s•L	182.3 ± 39.2	1219.3 ± 378,7*	166.7–500.1
Aspartate aminotransferase, nmol/s•L	180.6 ± 24.9	1200.2 ± 379.2*	166.7–833.5

Note: See Table 1.

In bulls of group II, the reaction to stress was sharper (Table 2). After a stress load, their concentration of cortisol exceeded the upper limit of the reference norm by 3.5 times, and testosterone by 1.7 times. After a stressful load (experiment), the concentration of cortisol exceeded the control by 287.2 nmol/L ($P<0.05$), and testosterone by 16.8 nmol/L ($P<0.05$), the activity of ALT increased by 1037.0 ($P<0.05$) and AST by 1019.6 nmol/s•L ($P<0.05$). The activity of creatine phosphate kinase compared to the reference norm increased slightly, but in the experiment the difference with the control was 650.1 nmol/s•L ($P<0.05$).
The results of the 24-hour timing of the behavior of breeding bulls are presented in the Table 3.

Table 3. The results of the daily timing of the behavior of bulls.

Indicator	Group	
	I, n=9	II, n=7
Food activity	751.3 ± 98.1	647.6 ± 60.3
including in the afternoon	428.8 ± 62.9	383.8 ± 60.1
at night	322.5 ± 54.5	263.8 ± 22.5
motor activity	878.8 ± 28.9	761.8 ± 37.7*
including in the afternoon	517.5 ± 33.1	451.3 ± 50.2
at night	361.3 ± 45.9	310.5 ± 39.8
Inactivity	435.3 ± 41.4	488.9 ± 54.8
including in the afternoon	200.1 ± 33.4	215.1 ± 38.1
at night	235.2 ± 64.2	273.8 ± 24.3
Rest lying down	720.8 ± 145.8	633.8 ± 166.4
including in the afternoon	195.6 ± 83.2	131.3 ± 77.9
at night	525.2 ± 85.5	502.5 ± 98.7
Rest during sleep	118.8 ± 30.9	125.3 ± 53.7
Comfortable actions	101.3 ± 29.3	83.9 ± 58.1
including in the afternoon	47.5 ± 14.4	60.1 ± 42.4
at night	53.8 ± 19.3	23.8 ± 16.5

Note: See Table 1.

An analysis of the results of timing observations indicates the relative rhythm of the daily regime of bulls. This is due to the comparatively identical conditions of maintenance and the routine of technological processes of feeding and removing manure. The nutritional activity in the bulls of group I was higher during the day by 44.5 minutes, at night by 58.7 minutes, and per day by 103.7 minutes. Motor activity, as a result of the general excitation of animals, reflects the physiological state, the level of stress of the body and is of great importance in the study of their behavior. During the whole experiment, the bulls of group I turned out to be more active. During the day, they moved longer than the servicing bulls of group II by 66.2, at night by 50.8 and during the day by 117 minutes ($P<0.05$). The alternation of rest and activity in both experimental groups during the day was almost the same. However, the bulls from group II stayed standing 53.6 minutes longer than from group I, including during the day by 15 minutes and at night by 38.6 minutes. The bulls of group I rested lying longer than their peers of group II during the day, the night and per day, respectively, by 64.3, 22.7 and 87 minutes. The sleep of the bulls took place from 22:00 pm to 6:00 am hours at intervals. Most of the animals rested from 23:00 pm to 4:00 am. The bulls of group II slept 6.5 minutes longer than the animals of group I. The bulls of group I spent 17.4 minutes more per day for comfortable activities. Functional bull behavior indices are presented in the Table 4.

Table 4. Functional indices of bull behavior.

Indicator	Group	
	I, n=9	II, n=7
Food index	0.52 ± 0.21	0.45 ± 0.12
Motor activity index	0.61 ± 0.0.02	0.53 ± 0.09*
Inactivity index	0.31 ± 0.03	0.34 ± 0.06
Comfort action index	0.07 ± 0.02	0.06 ± 0.07
Sleep index	0.17 ± 0.04	0.16 ± 0.09

Note: See Table 1.

The most pronounced difference between the bulls of the two groups is observed in terms of indices of motor and food activity, which were higher in animals of group I by 0.08 (P<0.05) and 0.07 units, respectively. The main indicators of sperm productivity and sperm quality are presented in the Tables 5–7.

Table 5. Indicators of bulls' sperm productivity for the first year of breeding use.

Indicator	Group	
	I, n=9	II, n=7
Ejaculate count	78.6 ± 19.93	58.3 ± 26.03
Ejaculate volume, ml	4.1 ± 0.65	3.7 ± 0.88
The number of sperm in the ejaculate, billion	4.1 ± 0.83	3.3 ± 0.58
Sperm suitable for freezing, ml	269.4 ± 86.06	141.4 ± 82.59*
Quality sperm doses of 1 ejaculate	94.8 ± 26.54	66.9 ± 21.68*
The concentration of sperm, billion/ml	1.1 ± 0.09	0.9 ± 0.08*
Sperm activity, points	7.9 ± 0.44	6.6 ± 1.09*
Fertilizing ability of sperm,%	68.7 ± 5.17	60.6 ± 6.12*
Sperm reject,%	12.8 ± 5.26	33.7 ± 9.63*

Note: See Table 1.

In the first year of breeding use, the sperm productivity of the bulls with different responses to stress differed sharply. Compared with group II, bulls of group I gave a larger number of ejaculates by 20.3. These bulls had more ejaculate volume on average by 0.4 ml, as well as the amount of sperm in the ejaculate by 0.8 billion, although the difference was unreliable. The reliable differences between the bulls of group I and group II were in the number of sperm suitable for freezing by 128 ml (P<0.05), in the number of qualitative sperm doses from one ejaculate by 27.9 (P<0.05), and the concentration of sperm cells by 0.2 billion/ml (P<0.05). Their semen was characterized by the best sperm activity by 1.3 points (P<0.05) and the fertilizing ability of sperm by 8.1% (P<0.05). The largest amount of sperm unsuitable for use was received from bulls of group II. Defect of their sperm compared with the peers of group I was higher by 22.2% (P<0.05).

Table 6. Sperm productivity of bulls for the second year of breeding use.

Indicator	Group	
	I, n=9	II, n=7
Ejaculate count	102.3 ± 26.78	91.8 ± 31.02
Ejaculate volume, ml	4.5 ± 0.88	3.7 ± 0.76
The number of sperm in the ejaculate, billion	4.9 ± 1.21	3.7 ± 0.57*
Sperm suitable for freezing, ml	409.5 ± 153.97	258.2 ± 94.43*
Quality sperm doses of 1 ejaculate	103.7 ± 31.75	69.5 ± 11.34*
The concentration of sperm, billion/ml	1.2 ± 0.08	1.1 ± 0.11
Sperm activity, points	8.1 ± 0.96	7.2 ± 1.18
Fertilizing ability of sperm,%	70.7 ± 5.22	63.1 ± 5.01*
Sperm reject,%	11.3 ± 6.16	24.2 ± 8.44*

Note: See Table 1.

In the second year of breeding, the sperm productivity of the bulls with different responses to stress also differed. Compared to group II, the bulls of group I gave by 10.5 more ejaculates. Their ejaculate volume was by 0.8 ml larger, and the sperm count in the ejaculate was by 1.2 billion more (P<0.05), the amount of sperm suitable for freezing by 151.3 ml (P<0.05), the number of quality sperm doses from one ejaculate by 34.2 (P<0.05), sperm concentration was higher by 0.1 billion/ml, sperm activity by 0.9 points and the fertilizing ability of sperm by 7.6% (P<0.05). The largest amount of low-quality sperm was obtained from the bulls of group II. Compared with the peers of group I, the rejection of their sperm was 12.9% higher (P<0.05).

Table 7. Indicators of breeding bulls' sperm productivity for the third year of use.

Indicator	Group	
	I, n=9	II, n=7
Ejaculate count	105.3 ± 35.72	89.1 ± 34.36
Ejaculate volume, ml	4.6 ± 1.13	4.1 ± 0.78
The number of sperm in the ejaculate, billion	5.1 ± 1.31	4.2 ± 0.72
Sperm suitable for freezing, ml	452.7 ± 242.97	298.2 ± 151.54
Quality sperm doses of 1 ejaculate	105.4 ± 33.96	76.8 ± 20.15
The concentration of sperm, billion/ml	1.2 ± 0.11	1.1 ± 0.07*
Sperm activity, points	8.3 ± 0.96	7.4 ± 1.59
Fertilizing ability of sperm,%	71.9 ± 5.25	62.8 ± 3.85*
Sperm reject,%	45.3 ± 30.48	69.5 ± 40.63

Note: See Table 1.

The indicators of the bulls' sperm productivity for the third year of breeding use were also different. It was received from the bulls of group I by 16.2 ejaculates more, with the volume higher by 0.5 ml, with the greater number of sperm in one ejaculate by 0.9. Their sperm was 1.5 times more suitable for freezing, the number of high-quality sperm doses from it was 1.4 times higher, and sperm activity was higher by 0.9 points. But the difference on these indicators was not reliable. The reliable predominant differences of the bulls from group I were found on the sperm concentration by 0.1 billion/ml (P<0.05) and sperm fertilization by 9.1% (P<0.05). Sperm rejection in bulls of group II exceeded the rate of bulls from group I by 1.5 times.

Discussion

Animals that produce more cortisol are more likely to experience short-term life shocks and unexpected troubles, but are more prone to chronic stress (Collier et al., 1982; Tsigos et al., 2002). The enzymes of alanine and aspartate aminotransferase in bulls play an important role in ensuring the redox and respiratory functions of sperm, as well as their capacitation. ATP resynthesis to restore homeostasis in the body depends on the activity of creatine phosphate kinase, and the effectiveness of this process can affect sperm quality and sperm motility, inhibition of biochemical processes in sperm, and their entry into suspended animation (Cordoba et al., 2007). Our data are consistent with these results. Although the activity of creatine phosphate kinase in bulls of group II increased slightly compared to the reference norm, but after a stress load the difference with the indicator before it was greater by 650.1 nmol/s•L (P<0.05), (whereas in bulls of group I it remained within the normal range), and the activity of ALT increased by 1037.0 (P<0.05) and AST by 1019.6 nmol/s•L (P<0.05).

In our studies, a higher percentage of semen rejection in bulls of group II can be explained by the fact that under the conditions of a stress reaction, an excess of lactic acid is formed in the body due to which sperm are damaged and die, or their suspended animation occurs, gamete activity is lost (Brito et al., 2002). In addition, cortisol, retaining energy in the body to overcome stress, inhibits the effects of testosterone and the processes of biochemical synthesis in tissues (Welsh & Johnson, 1981; Wilkerson et al., 2016). A young organism is more sensitive to different operational loads than an adult organism (Al-Kanaan et al., 2015). Until the age of 12 months, the formation of the type of nervous system in bulls is only completing. (Nikitchenko et al., 1988) because of which they are vulnerable to various stress factors. And in our studies, sharper differences in sperm productivity were discovered between the bulls of the opposite groups precisely in the first year of their breeding use.

The results of our observations of the behavior of bulls are consistent with the data (Velikzhanin, 1995), which established that in animals of a weak type of the nervous system, motor reactions last for the shortest time, while inhibitory ones take the greatest amount of time. They have 80% of the time of inhibitory reactions account for inactivity while standing and only 18% of the time for rest lying down. In our studies, the inactivity index was by 12.3% higher in bulls of group II than in their peers with higher adaptive abilities. The bulls of group I rested lying longer than peers of group II an hour and a half. The bulls of group II, on the contrary, were in a standing position for almost an hour longer than the bulls of group I. And if the animal does not sufficiently rest lying, then this can additionally increase the level of stress in its body and worsen its well-being (Frondelius et al., 2015). In the conditions of the breeding station where our studies were conducted, boxes for keeping the animals of a weaker type are located between the boxes of bulls of a stronger type of the nervous system, which, in our opinion, can also negatively affect their well-being. We intend to expand future studies of the welfare of bulls by using new technologies for automated monitoring of animal behavior and applying digital infrared thermography of the scrotum, taking into account global climate change.

Conclusion

The general welfare of bulls depends on their resistance to stress. More resistant to operational loads bulls are characterized by greater activity in the manifestation of any behavioral acts during the day. In contrast, bulls of group II turned out to be more passive, moved less, consumed less food, and remained inactive for longer. For the first year of use, compared with the group II, stress-resistant bulls received significantly more sperm suitable for freezing (P<0.05), higher the total number of quality sperm doses from one ejaculate (P<0.05), with a higher concentration of sperm (P< 0.05), the best sperm activity (P<0.05) and the fertilizing ability of sperm (P<0.05). Similar results are observed for the second and the third years of use. The largest amount of sperm not suitable for use was obtained from bulls of group II. Compared to peers of group I, their semen rejection was higher by 22.2% (P<0.05) in the first year, by 12.9% (P<0.05) in the second year and by 24.2% in the third year of use. Thus, the bulls with high stress resistance have the best overall well-being of the body and from them it is guaranteed faster to accumulate the necessary bank of high-quality sperm.

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