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ORIGINAL ARTICLE

Specific and nonspecific resistance of the organism and sperm indicators of breeding stallions in Ukraine to the influence of feed mycotoxins

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The article presents studies of the permissible concentrations of feed mycotoxins influence on the indicators of specific and nonspecific organism resistance of breeding stallions of Ukrainian riding, Trakehner, Westphalian and Arabian breeds bred in Ukraine. In Arabian stallions, decreased: blood serum bactericidal activity by 20.8% ($p<0.001$); the lysozyme blood serum activity by 2.3% ($p<0.001$); the phagocytosis completeness index by 32.3% ($p<0.001$), the relative number of lymphocytes by 23.1% ($p<0.001$). This caused an increase: in their total bacterial contamination of native sperm by 4079 colony-forming units per cm^3 ($p<0.001$); thawed sperm by 10473 colony-forming units per cm^3 ($p<0.001$); in micromycete contamination of native sperm by 36.1 colony-forming units per cm^3; thawed sperm by 121.6 colony-forming units per cm^3 ($p<0.001$) and a decrease in the biotechnological suitability of sperm by 54.2%.

Keywords: Feed mycotoxins, resistance, stallions, semen contamination.

Introduction

According to official statistics, the yield of foals in the whole horse breeding industry in Ukraine does not exceed 48%. Therefore, the primary scientific and practical task is to increase the yield of foals, which cannot be solved without an integrated approach.

It is possible to raise the yield of foals through the widespread introduction of modern methods of reproduction and biotechnology into practice. However, it is necessary to consider factors that may reduce their effectiveness from immunogenetic incompatibility in the stallion-mare-fetus system (Tkachev, 2013; Tkachev et al., 2020; Kovalenko et al., 2020). Concomitant secondary diseases can cause embryonic mortality (for example, Salmonella abortion and others), bacterial and micromycete contamination of stallion sperm (7-8), and specific and nonspecific resistance of the horse's organism.

The effectiveness of horse reproduction biotechnology methods also largely depends on the initial quality of the native sperm of breeding stallions and its contamination with bacterial and fungal microflora. The primary source of stallion sperm defilement is the contamination of the preputial cavity, which may depend on the nonspecific and specific resistance of the organism. Mycotoxins have a depressing effect on nonspecific and specific resistance (Paterson & Lima, 2010; Santos Pereira et al., 2011; Streit et al., 2012; Rai et al., 2015; Cimbalo et al., 2020).

The problem of high contamination of concentrated feed of horses with mycotoxins is widely known globally and in Ukraine (Tsvilikhovsky et al., 2010; Brezvyn et al., 2013; 2021; Vasylyev et al., 2021). The ability of mycotoxins to accumulate in the body and thus have a delayed negative impact (Filannino et al., 2011; Alassane-Kpembi et al., 2017; Tkachev et al., 2018; Roman et al., 2020), low knowledge of the effect of permissible levels of feed mycotoxins on homeostasis, resistance, and immune status of horses (Diaz, 2006) have been explored. Therefore, in the conditions of horse breeding in Ukraine, experiments on the influence of secure concentrations of feed mycotoxins on the indicators of specific and nonspecific resistance of the organism of breeding stallions connected with bacterial and micromycete contamination of their sperm are of particular importance.

That is why it is essential to study the relationship between the acceptable concentrations of mycotoxins and the organism resistance of breeding stallions and the contamination of their sperm.

The research aims to establish the influence of the maximum secure concentrations of feed mycotoxins on the indicators of specific and nonspecific resistance of the organism of breeding stallions of factory breeds of Ukraine in connection with bacterial and micromycete contamination of their sperm.

Materials and Methods

The investigation was carried out in Ukraine on seven breeding stallions of the Ukrainian horse breed of the Kharkiv stud farm, five stallions-producers of the Trakehner breed, and three breeding stallions of the Arab breed of the Chutovsky stud farm "Traken" of the Poltava region, on five stallions-producers of the Westphalian breed of the Petrekovsky stud farm of the Dnepropetrovsk region**.** According to the Kharkiv technology developed by the laboratory of artificial insemination, obtaining and cryopreservation of stallion sperm was carried out according to the laboratory of artificial insemination, IAH NAAN (Sushko et al., 2006). The number of colony-forming units (CFU) of bacterial and micromycete contamination of sperm was identified by the generally accepted methods GOST 20909.2-75 and GOST-10444.12-88, respectively. The contamination of feed with mycotoxins was determined at the Laboratory of Mycotoxicology IAH NAAS, according to which the maximum secure concentrations of mycotoxins (up to 0.1 mg/kg of T-2 toxin, 0.08-1.0 mg/kg of zearalenone, up to 1.0 mg/kg deoxynivalenol, 0.04 mg/kg aflatoxin). Standard methods in the blood were identified: bactericidal activity of blood serum (BASK), lysozyme activity of blood serum (LASK), index of completeness of phagocytosis (IPF), the number of T-and B-lymphocytes (Ionov al., 2011). Statistical processing of the results was carried out by conventional methods using the specialized program SPSS (Plokhinsky, 1969).

Results and Discussion

At the first stage of the investigation, the indices of native sperm of stallions of the Ukrainian riding, Hanoverian, Thoroughbred riding, and Trakehner breeds were established, including general bacterial and micromycete contamination (Table 1).

Table 1 shows that the quantitative and qualitative indices of native sperm were the best in Arabian stallions. At the same time, the total bacterial contamination was the highest in Arabian stallions, which is 3046.0 colony forming units (GBC), more than the maximum allowable level. The slightest micromycete contamination of native sperm was observed in Trakehner stallions.

The volume of ejaculate, the activity, and concentration of sperm, the number of pathological forms of sperm significantly worsened, and the bacterial and micromycete contamination of native sperm significantly increased after 5-6 weeks of administrating an acceptable level of mycotoxins (Table 1).

Indicators	Ukrainian horse	Trakehner	Arabic	Westphalian
Before receiving feed with mycotoxins				
Number of ejaculates received	35	25	14	26
Ejaculate volume, cm^3	59.80 ± 4.46	69.37 ± 7.49	21.80 ± 1.97	74.92 ± 5.42
Native sperm activity, points	5.47 ± 0.22	5.58 ± 0.13	7.40 ± 0.22	6.00 ± 0.21
Sperm concentration, mln/cm^3	113.97 ± 6.65	95.84 ± 5.27	207.8 ± 10.43	120.08 ± 5.69
Pathological forms of sperm, %	15.94 ± 0.27	15.96 ± 0.59	18.20 ± 0.33	12.64 ± 0.54
General bacterial contamination, GBC/cm^3	2774.6 ± 257.8	2376.4 ± 209.5	8046.0 ± 332.7	2856.8 ± 188.5
General micromycete contamination, GMC/cm^3	71.72 ± 3.02	32.67 ± 6.65	106.67 ± 17.06	45.32 ± 6.69
5-6 weeks after receiving mycotoxin feed				
Number of ejaculates received	56	41	24	39
Ejaculate volume, cm^3	48.66 ± 3.11*	54.77 ± 4.94	17.94 ± 1.46	59.45 ± 4.,09*

Native sperm activity, points	4.89 ± 0.12*	4.88 ± 0.07***	6.38 ± 0.13**	5.25 ± 0.20*
Sperm concentration, mln/cm^3	95.32 ± 4.22*	83.48 ± 3.74	168.75 ± 14.6*	104.38 ± 3.45*
Pathological forms of sperm, %	18.38 ± 0.2***	20.6 ± 0.64***	22.0 ± 0.68***	22.58 ± 0.93***
General bacterial contamination, GBC/cm^3	4580.5 ± 296.0 ***	6941.0 ± 292.1 ***	12125.0 ± 357.0 ***	4339.0 ± 446.8 **
General micromycete contamination, GMC/cm^3	11666 ± 6.30***	57.99 ± 5.23**	142.75 ± 14.46	67.48 ± 6.19*

Note: *-p<0.05; **-p<0.01; ***-p<0.001.

Table 1. Native semen indicators of different breed stallions before and after receiving feed with an acceptable level of mycotoxins (M ± m).

The biotechnological suitability of the semen of stallions of the studied breeds was at a reasonably high level from 80 to 100% (Table 2) before receiving feed with mycotoxins. At the same time, bodily contamination was upper than the permissible level only in the semen of Arabian stallions.

Indicator	Ukrainian horse	Trakehner	Arabic	Westphalian
Before receiving feed with mycotoxins				
Number of ejaculates received	35	25	14	26
The number of ejaculates suitable for freezing	29	25	14	24
Biotechnological suitability, %	82.86	100.0	100.0	92.3
Sperm activity, points	3.24 ± 0.16	4.32 ± 0.11	5.90 ± 0.10	4.92 ± 0.15
Sperm survival at 37 °C, hours	3.36 ± 0.17	3.88 ± 0.17	5.30 ± 0.21	5.44 ± 0.26
Sperm preservation,%	46.25 ± 4.21	77.72 ± 1.63	80.36 ± 2.78	82.48 ± 1.36
General bacterial contamination, GBC/cm^3	3224.3 ± 292.4	3658.0 ± 252.6	9777.0 ± 105.1	3271.4 ± 243.8
General micromycete contamination, GMC/ cm^3	85.77 ± 3.87	45.96 ± 9.17	202.33 ± 23.79	78.56 ± 11.56
5-6 weeks after receiving mycotoxin feed				
Number of ejaculates received	56	41	24	39
The number of ejaculates suitable for freezing	34	23	11	28
Biotechnological suitability, %	60.71	56.1	45.83	71.8
Sperm activity, points	2.82 ± 0.09*	2.33 ± 0.05***	3.5 ± 0.18***	2.92 ± 0.13***
Sperm survival at 37 °C, hours	2.79 ± 0.08**	2.04 ± 0.16***	1.53 ± 0.20***	2.69 ± 0.20***
Sperm preservation,%	38.41 ± 4.25	47.75 ± 0.76***	55.5 ± 2.85***	52.14 ± 4.24***
General bacterial contamination, GBC/cm^3	7013.9 ± 319.3 ***	8574.3 ± 158.0 ***	20250.0 ± 567.0 ***	13363.0 ± 446.0 ***

General micromycete contamination, GMC/ cm^3	204.41 ± 12.03***	114.55 ± 9.36***	323.88 ± 25.10**	128.65 ± 11.40**

Note: *-p<0.05; **-p<0.01; ***-p<0.001.

Table 2. Thawed semen parameters of different breed stallions before and after receiving feed with an acceptable level of mycotoxins (M ± m).

Micromycete contamination of thawed sperm was the highest in Arabian stallions, which is 57.6% more (p<0.001) from the Ukrainian riding breed, 77.3% further (p<0.001) than the Trakehner breed, and 61.2% more (p <0.001) of Westphalian breeders.

After receiving feed with the maximum permissible level of zearalenone, T-2 toxin, deoxynivalenol, and aflatoxin, the semen of Ukrainian riding stallions' biotechnological suitability decreased by 22.2%, Trakehner-by 43.9%, Arab-by 54.2%, and Westphalian-by 20.5%. At the same time, the activity, vitality, and safety of sperm cells after thawing significantly worsened. Bacterial contamination of thawed semen of Ukrainian riding stallions elevated 2.2 times (p<0.001), Trakehner-2.3 times (p<0.001), Arabian-2.1 times (p<0.001), Westphalian-4 , 1 time (p<0.001). Micromycete contamination of thawed semen of stallions of the Ukrainian riding breed increased 2.4 times (p<0.001), Trakehner-2.5 times (p<0.001), Arabian and Westphalian breeds-1.6 times (p<0.01).

The established deterioration of native and thawed sperm indices, its biotechnological suitability, and progress in bacterial and micromycete contamination occurred against the background of significant deterioration in the indices of resistance of the organism of breeding stallions (Table 3).

Indicator	Ukrainian horse	Trakehner	Arabic	Westphalian
Before receiving feed with mycotoxins				
Number of samples	35	25	14	26
BASK, %	65.06 ± 0.48	87.84 ± 1.12	71.77 ± 0.56	78.44 ± 1.39
LASK, %	22.26 ± 0.34	22.53 ± 0.35	17.60 ± 0.19	20.13 ± 0.29
PCI, units	0.78 ± 0.00	0.83 ± 0.01	0.93 ± 0.01	0.88 ± 0.01
Lymphocytes, %	36.14 ± 0.71	38.24 ± 0.64	41.70 ± 0.45	40.16 ± 0.61
T-lymphocytes, 10^9/l	2.55 ± 0.08	2.88 ± 0.07	3.73 ± 0.08	3.49 ± 0.10
B- lymphocytes, 10^9/l	0.70 ± 0.02	0.64 ± 0.01	0.66 ± 0.03	0.60 ± 0.02
5-6 weeks after receiving feed with acceptable levels of mycotoxins				
Number of samples	56	41	24	39
BASK, %	55.21 ± 0.43***	61.08 ± 0.58***	50.98 ± 0.24***	71.03 ± 1.02***
LASK, %	18.64 ± 0.32***	19.14 ± 0.17***	15.33 ± 0.08***	16.77 ± 0.24***
PCI, units	0.72 ± 0.01***	0.74 ± 0.01***	0.63 ± 0.01***	0.71 ± 0.01***
Lymphocytes, %	22.70 ± 0.42***	22.98 ± 0.35***	18.63 ± 0.40***	25.60 ± 1.04***
T-lymphocytes, 10^9/l	1.03 ± 0.04***	1.18 ± 0.02***	0.70 ± 0.02***	1.57 ± 0.11***
B-lymphocytes, 10^9/l	0.26 ± 0.01***	0.26 ± 0.01***	0.14 ± 0.01***	0.27 ± 0.02***

Note: ***-p<0,001.

Table 3. Natural resistance indicators of stud stallions of different breeds, stallions before and after receiving feed with an acceptable level of mycotoxins (M ± m).

From the data in Table 3, it can be seen that on the background of an increase in bacterial and micromycete contamination of the semen of stallions of the Ukrainian riding breed, the bactericidal activity of blood serum (BASK) deteriorated by 9.9% ($p<0.001$), lysozyme activity of blood serum (LASK)-by 3.6% ($p<0.001$), phagocytosis completeness index (IPF)-by 7.7% ($p<0.001$), the total relative number of lymphocytes-by 13.4% ($p<0.001$), the number of T-lymphocytes-by 2.5 times ($p<0.001$), the number of B-lymphocytes-2.7 times ($p<0.001$). Similar changes in the indices of natural resistance were observed in other breeds. However, Arabian stallions were most sensitive to long-term feed intake with the maximum allowable zearalenone levels, T-2 toxin, deoxynivalenol, and aflatoxin.

Correlation analysis of variance showed that the bactericidal activity of blood serum (BASK) has a significant correlation with total bacterial contamination of native sperm minus 0.29 ($p<0.01$), with micromycete contamination of native sperm-minus 0.64 ($p<0,01$); lysozyme activity of blood serum (LASK) correlates with total bacterial contamination of native sperm-minus 0.61 ($p<0.01$), with micromycete contamination of native sperm-minus 0.34 ($p<0.01$); phagocytosis completeness index (IPF) correlates with total bacterial contamination of native sperm-0.26 ($p<0.05$), with micromycete contamination of native sperm-minus 0.13.

Conclusion

For the first time in Ukraine, we studied the influence of the maximum permissible levels of zearalenone, T-2 toxin, deoxynivalenol, and aflatoxin in the feed on the resistance of stallions-producers of Ukrainian horse, Trakehner, Arabian and Westphalian breeds toward bacterial and micromycete contamination of their sperm. After 5-6 weeks of feeding the stallions with food with the maximum allowable levels of zearalenone, T-2 toxin, deoxynivalenol, and aflatoxin, the worst indicators of natural resistance were found in Arabian breeders. In Arabian stallions, the bactericidal activity of the blood serum decreased by 20.8% ($p<0.001$), the lysozyme activity of the blood serum-by 2.3% ($p<0.001$), the phagocytosis completeness index-by 32.3% ($p<0.001$), the relative number of lymphocytes-by 23.1% ($p<0.001$), which may have caused an increase in their total bacterial contamination of native sperm by 4079 colony-forming units per cm^3 ($p<0.001$), thawed sperm-by 10473 colony-forming units per cm^3 ($p<0.001$); an increase in micromycete contamination of native sperm by 36.1 colony-forming units per cm^3, thawed sperm-by 121.6 colony-forming units per cm^3 ($p<0.001$) and a decrease in the biotechnological suitability of sperm by 54.2%.

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