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Indicators of reproduction when using complex use of drugs for spontaneous manifestation of heat in cows for mycotoxicosis

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Abstract. Alimentary infertility is becoming increasingly important due to the toxic effects of mycotoxins, especially zearalenone and deoxynivalenol, which leads to a decrease in the efficiency of the cattle breeding industry, so it is necessary to examine this problem. The purpose of the study was to identify the effect of the complex use of sorbents, acidifiers, and aromatase inhibitors on the reproductive capacity of cows in mycotoxicosis. During the study, clinical methods (general examination) and biochemical methods (serum content of calcium, magnesium, urea, total and direct bilirubin, malonic

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dialaldehyde, aminotransferase activity) were used. Cows were divided into two groups: the first group was a control group, where no treatment was used, and cows of the second group received a comprehensive treatment method. A decrease in the level of calcium to 1.68 ± 0.53 mmol/L, phosphorus – 0.98 ± 0.16 , selenium – 0.64 ± 0.1 mmol/L, an increase in the level of magnesium to 1.15 ± 0.17 mmol/L, an increase in aspartate aminotransferase to 121.33 ± 3.91 units, alataminotransferase to 79.31 ± 6.53 units, and total bilirubin to 10.21 ± 0.55 mmol/L, malone aldehyde up to 8.27 ± 0.41 mmol/L, and ceruloplasmin up to mmol/L 2.14 ± 0.39 in chronic mycotoxicosis caused by zearalenone and deoxynivalenol. When using a complex treatment regimen for animals, a substantial change and approximation to the reference levels of selenium, creatinine, AST, ALT, malonic aldehyde, and ceruloplasmin were established. The use of the proposed treatment regimen increases the fertilising capacity of cows after the 1st calving by 18.15%, cows aged 3-4 years – 30.12%, and cows of the older age group – by 40.47%. A complex method of treatment of cows for mycotoxicosis caused by zearalenone is proposed using an acidifier based on organic acids, a sorbent based on celeolite and an aromatase inhibitor, which can be used in livestock farms for the production of milk and meat of various forms of ownership to restore and normalise the reproductive ability of cows

Keywords: mycotoxins; reproduction; aromatase inhibitor; antioxidant protection

INTRODUCTION

One of the widespread problems in cattle breeding is the use of concentrated and coarse feed affected by mycotoxins, in particular, the *Fusarium* genus. The low reproductive capacity of cows causes substantial economic damage to livestock farms due to overspending of feed on infertile animals, premature culling, early abortions, and germ resorption, which leads to an increase in the cost of products created, and a decrease in the competitiveness of enterprises. Therefore, improving the methods of reproduction of cows remains the main task of veterinary specialists in farms. Due to the use of animals and sperm imported from abroad, the quality of feed and the requirements for keeping breeding stock are becoming increasingly important.

Therewith, some studies report the development of destructive changes in the ovaries, uterus (Bailey *et al.*, 2019), kidneys, liver (Yang *et al.*, 2020). Authors S. Fedorenko *et al.* (2017) and L. Silva *et al.* (2021) argue that the use of feed with a high content of zearalenone leads to an increase in the level of estrogen-like substances, the main of which are β -searalenol, α -searalenol in a ratio of 1:11, but the author provides data at laboratory-created levels of zearalenone, but the study remains relevant in production conditions when the level of individual mycotoxins is due to accumulation during harvesting, storage, and preparation of feed for consumption. Researchers E. Lee *et al.* (2021) report that as a result of chronic poisoning caused by β -searalenol, follicular cysts occur in the ovaries of cows due to a violation of the synthesis of follicle-stimulating and luteinising hormones due to inhibition of the formation of the α -isomer of releasing hormone, with luteinising hormone-sensitive receptors playing a leading role, and this study is aimed at restoring the balance of follicle-stimulating and luteinising hormones. In the paper by G. Zhang *et al.* (2018), it is reported that in chronic mycotoxicosis, the formation of sex hormones is suppressed due to destructive changes in the ovaries, which manifest themselves in the form of optosis of cells of the ovarian cortical layer.

Similar results were obtained by other researchers, M. Abdel-Kareem *et al.* (2019), indicating a negative effect of mycotoxins on the reproductive capacity of cows and heifers of mating age. The authors propose a large number of means to reduce the negative impact of mycotoxins on the body of cows, namely preservatives in the collection and storage of feed, sorbents in the preparation of feed and hepatoprotectors in the treatment of cows with mycotoxicosis. However, as noted by L. Silva *et al.* (2021), long-term use of feed containing zearalenone leads to ovarian dysfunction due to apoptosis of cortical layer cells and organisation of brain and mezentorial layer cells. H Kadokawa (2020) provides data confirming the violation of cyclical changes in the types of endometrium of the uterus during the sexual cycle, and therefore there is an imbalance of hormones: sex – estradiol, progesterone and those that are formed in the hypothalamus – releasing factor (α - and β -isomers), and pituitary-follicle – stimulating and luteinising, as indicated by another researcher.

The purpose of the study was to investigate the effectiveness of using a comprehensive treatment regimen for cows with mycotoxicosis using certain types of celeolites, organic acids, and lipid oxidation inhibitors.

MATERIALS AND METHODS

The study was conducted in milk production farms Agrofirma "Lan", Agrofirma "Mriya", and Limited Liability Company Agrofirma "Vladana", Sumy region during 2019-2021. In each farm, the number of cows was examined and a diagnosis of mycotoxicosis was established. Then the cows were divided into two groups (in each farm). In the first experimental group of cows, a complex application of biological agents was used to reduce the effect of zearalenone on the reproductive ability of cows (an acidifier based on organic acids, a sorbent based on celeolite, and an aromatase inhibitor). The acidifier was added to drinking water, creating a concentration of 0.1%, which was given to the cows for 5 days. Celeolite-based sorbent was used at the rate of 2.5 kg per

1 ton of feed for 14 days. An aromatase inhibitor (letrozole) was used at a dose of 30 mg once, orally, when the dominant follicle reached a diameter of 18 mm. In the second group (control), no funds were used.

Therewith, the types of ovarian and uterine pathology were considered (they were established by transrect examination using a sensor with a Convex matrix of 6-9 MHz (Ultrasonix RP). The dynamics of some biochemical parameters in all experimental groups is established. Therewith, generally accepted methods were used to determine calcium by reaction with Arsenazo III, magnesium – by reaction with xylydyl blue, activity of aminotransferases (AST, ALT) – kinetic method (in vitro), activity of aspartate aminotransferase (AST) – kinetic method (in vitro), urea – urease method; total and direct bilirubin (according to the Endrashik method); activity of γ -lutamyltransferase – with the substrate L- γ -glutamyl – p-nitroaniline, malone dialaldehyde (MDA) was performed spectrophotometrically. The next step was to determine the effectiveness of the use of biologically active agents on reproductive ability. Therewith, fertilisation indicators, insemination index, and hidden abortions were established.

All experimental studies were conducted in accordance with modern methodological approaches

and in compliance with the relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006). The keep of animals and all manipulations were conducted in accordance with the provisions of the procedure for conducting experiments and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

RESULTS AND DISCUSSION

Biochemical parameters are markers of pathological changes both in the body as a whole and in individual organs and systems. Some biochemical parameters of the blood were examined, indicating both metabolic disorders (calcium, phosphorus and magnesium), toxic effects of mycotoxins (AST, ALT, total bilirubin), and violations of antioxidant protection in violation of lipid oxidation under the influence of zearalenone (selenium, malonic aldehyde Te ceruloplasmin) to diagnose and predict the occurrence of gynecological pathology. The results of studies of changes in individual biochemical parameters of cow blood for mycotoxicosis are presented in Table 1.

Table 1. Comparison of biochemical parameters of blood in cows for mycotoxicosis

Indicator	Control group (n=12)	Cows after applying acidifier (n=12)	reference indicators	P
Calcium, mmol/L	1.68±0.53	2.23±0.31	2.4-2.6	0.38
Phosphorus, mmol/L	0.98±0.16	1.2±0.34	1.45-2.1	0.556
Magnesium, mmol/L	1.15±0.17	0.89±0.13	0.8-1.15	0.246
Selenium, mmol/L	0.64±0.1	1.1±0.15*	0.8-1.9	0.018
Creatinine, nmol/d	107.3±4.59	82.6±3.5**	80.0-130.0	0.001
AST	121.33±3.91	67.25±7.61**	10.0-50.0	0.001
ALT	79.31±6.53	37.28±4.12**	10.0-30.0	0.001
De Ritis Indicator	1.53	1.8	1-1.5	
Bilirubin, μ mol/L	10.21±0.5	7.65±1.13	0.3-7.0	0.061
Malonic aldehyde, mmol/L	8.27±0.41	5.27±0.69*	4.5-6.0	0.002
Ceruloplasmin, μ mol/L	2.14±0.39	1.69±0.47	1.6±2.0	0.393

Note: * – $p < 0.05$, ** – $p < 0.001$

Source: compiled by the authors

Therewith, a positive increase in the use of a complex treatment regimen for cows with mycotoxicosis is shown. When using an acidifier based on organic acids together with a sorbent based on celeolite and an aromatase inhibitor, a result was obtained indicating the restoration of calcium levels, while in the control group (cows in whose diet the amount of zearalenone exceeded 400 mg/kg) it remained at the level of 1.68±0.53 mmol/L. In addition, when exposed to mycotoxins, in particular,

deoxynivalenol, magnesium levels were recorded at the upper limit of the reference limit, which also contributed to a decrease in calcium levels and an imbalance of calcium, phosphorus, and selenium. There is evidence indicating a correlation between selenium levels and the fertilisation capacity of cows.

The use of the proposed treatment regimen for cows under the influence of zearalenone and deoxynivalenol contributed to a decrease in malone aldehyde

by 1.57 times and ceruloplasmin – by 1.27 times. Notably, these indicators have restored their level to the same level in healthy animals. In addition, the proposed scheme helped to reduce toxic effect on the body of cows. This is indicated by a decrease in AST

by 1.8 times and ALT by 2.13 times, which is confirmed by studies by other authors (Kinoshita *et al.*, 2018). Reducing the toxic effect and restoring the antioxidant protection of the cow's body led to an increase in reproductive capacity (Table 2).

Table 2. Indicators of reproduction when using a complex treatment regimen for cows with mycotoxicosis

Indicators	Without treatment			Using a comprehensive treatment regimen		
	Cows, 1st calving	Cows aged 3-4 years	Cows aged 5-7 years	Cows, 1st calving	Cows aged 3-4 years	Cows aged 5-7 years
Number of animals	102	114	112	98	124	107
Duration of the postpartum period	40.1±0.22	45.3±1.21	52.9±2.67	32.8±2.39*	37.4±3.68	39.1±3.67*
Duration of the service period	70.2±1.18	73.61±1.97	73.39±2.63	52.39±2.61 *	55.97±2.67*	59.67±2.32*
Number of calves per 100 cows	82.64	79.42	72.39	93.41	90.37	85.94
Insemination index	3.28±0.18	3.41±0.22	3.68±0.31	2.1±0.21	2.4±0.18	2.84±0.19
Rejected for various reasons	14	32	38	10	14	16

Note: * – $p < 0.001$

Source: compiled by the authors

A substantial decrease in the duration of the postpartum period was identified in cows after the first calving compared to animals of the same age group with mycotoxicosis. The tendency to reduce the postpartum period was also observed in older age groups, but it was statistically unreliable. This is due to deeper destructive changes in the organs of the reproductive system in cows, in particular, the uterus (thickening of the uterine mucosa and organisation processes) with chronic exposure to zearalenone and apoptosis processes in the ovarian tissue, which in turn inhibited the processes of involution of the genitals of cows after childbirth.

In general, zearalenone contributed to a constant increase in the level of estrogen-like substances in the blood of cows, which led to the development of destructive changes in the endometrium of the uterus. The use of a complex treatment regimen using an acidifier, sorbent, and aromatase inhibitor helped to reduce the toxic effect of zearalenone on the endometrium, ovaries and increase the antioxidant capacity of cows. Positive dynamics were established in all age groups of such indicators as the number of offspring received and the insemination index. The main indicator of restoration of reproductive ability is fertilisation. The analysis of cow fertilisation in a comparative aspect is conducted (Table 3).

Table 3. Fertilisation by day 120

Indicators	Without treatment						Using a comprehensive treatment regimen					
	Cows, 1st calving		Cows aged 3-4 years		Cows aged 5-7 years		Cows, 1st calving		Cows aged 3-4 years		Cows aged 5-7 years	
	number	%	number	%	number	%	number	%	number	%	number	%
Number of animals	102		114		112		98		124		107	
Total fertilised	71	69.61	53	46.49	29	25.89	86	87.76	95	76.61	71	66.36
Not fertilised	31	30.39	61	53.51	83	74.11	12	12.24	29	23.39	36	33.64

Note: * – $p < 0.05$, ** – $p < 0.001$

Source: compiled by the authors

Therewith, the fertilisation rate of cows after the 1st calving after applying a comprehensive treatment regimen increased 1.26 times (18.15%), among cows aged 3-4 years – 1.65 times (30.12%). Cows of the older age group after using the proposed scheme had fertilisation by 2.56 times more often (40.47%). This difference is explained by the development of hypogonadism in

older cows, characterised by a large number of atretic follicles and the absence of dominant follicles due to the chronic effect of zearalenone on the ovaries of cows. This is confirmed by the study by J. Gong *et al.* (2020). The use of an aromatase inhibitor reduces the level of lipid peroxidation and the release of estrogen-like substances, which in turn can reduce the amount of

follicle-stimulating and luteinising hormones, which leads to defective sexual cycles, in particular, anovulatory ones. In chronic poisoning with mycotoxins, in particular, zearalenone and deoxynivalenol, a huge number of both compensatory and destructive mechanisms occur in the body. Certain biochemical parameters are markers of such changes, so their interpretation is fundamental in the diagnosis and development of methods for the prevention and treatment of cows exposed to mycotoxins in general and zearalenone in particular.

A decrease in the level of calcium in cows under the influence of mycotoxins occurs firstly, as a consequence of the use by the cow's body of a large amount of this macronutrient in a cascade of redox reactions under oxidative stress of the body, and secondly-poor absorption of calcium due to destructive changes in the intestinal wall. This statement is supported by authors I. Alassane-Kpembi *et al.* (2019), indicating a decrease in the viability of intestinal cells and the concentration of short-chain fatty acids, and as a result, the death of saprophytic bacteria that counteract oxidative stress and impair effective digestion and absorption of substances. Selenium is known to play a role in preventing oxidative stress in the body and apoptosis of ovarian cells and stimulating the formation of blastocysts in the embryo (Gonzalez Andueza *et al.*, 2022). Most authors tend to believe that a decrease in selenium levels under the action of mycotoxins leads to a decrease in the overall antioxidant capacity, while the level of malonic aldehyde and ceruloplasmin increases (Gong & Xiao, 2018).

After applying a complex treatment regimen for cows with chronic mycotoxicosis, the amount of selenium increased, which is associated with the restoration of redox reactions in the body due to the blocking of aromatase and slowing down the formation of estrogen-like substances, including from adipose tissue. A similar trend has been identified by A. Iorga *et al.* (2017), indicating a positive effect of aromatase in the development of oxidative stress. Another diagnostic criterion for the development of oxidative stress, and therefore a violation of the synthesis of both gonadotropic hormones and sex hormones, is the level of malone aldehyde. Thus, a decrease in the level of malone aldehyde in the blood serum to the reference values was observed after the use of the proposed treatment of cows. It is known that in infectious diseases and toxicoses caused by mycotoxins and bacteria, the amount of malone aldehyde increases (Ayemele *et al.*, 2021). Therefore, it is possible to start the idea of a positive result of using the proposed treatment for oxy-reducing reactions in chronic mycotoxin poisoning.

There was a reduction in the duration of the postpartum period after the use of a complex treatment regimen with a sorbent based on celeolite, an oxidant based on organic acids and an aromatase inhibitor, which is associated with the restoration of the synthesis of follicle-stimulating hormone and prostaglandin

F_{2A} uterine tissues, which stimulated the contraction of the uterus, and then the processes of involution of the organs of the reproductive system. Therewith, the yellow body of pregnancy melts, the amount of progesterone decreases sharply and the local protective reaction of the endometrium increases. Other authors agree with these statements, in particular, M. Adnane & A. Chapwanya (2022) indicate an increase in the number of lactic acid bacteria on the vaginal mucosa, which in the process of vital activity produce lactic acid, which acts bactericidal on conditionally pathogenic microflora that entered the organs of the reproductive system during childbirth.

Special attention should be paid to the duration of the service period because it directly affects the effectiveness of insemination at the first manifestation of the stage of sexual heat arousal in cows. At this time, it is important to establish the condition of the organs of the reproductive system of cows before insemination. This is due to the fact that according to foreign authors (Wagener *et al.*, 2017; Ballas *et al.*, 2022; Pascottini *et al.*, 2023), during this period subclinical inflammatory processes are diagnosed, namely endometritis, salpingitis, or ovariitis. After applying the prescribed treatment, the duration of the service period decreased by an average of 25%. This is due to a faster involution of the organs of the reproductive system, after which dominant follicles are formed in the ovary, and therefore the first ovulation after childbirth. This standpoint is supported by Y. Lin *et al.* (2021). The presented data indicate substantial changes in the biochemical parameters of cow blood due to the negative effect of mycotoxins, which negatively affect the reproductive capacity. A characteristic complex change in the main biochemical parameters is both a diagnostic and prognostic test for the occurrence of infertility due to exposure to zearalenone and deoxynivalenol. The proposed treatment regimen for sick cows can be used to treat cows with mycotoxicosis to restore and stimulate the reproductive ability of cows and heifers.

CONCLUSIONS

Long-term feeding of feed containing zearalenone leads to a decrease in the reproductive capacity of cows after the 1st calving by 30%, cows of the middle age group (3-4 years) – by 53.5%, and cows of the older age group – by 74.11%. The presence of zearalenone in the diet of more than 400 mg/kg contributes to the accumulation of estrogen-like substances and a decrease in the level of calcium, phosphorus, and the antioxidant capacity of the cow's body. The use of complex treatment of cows using an acidifier based on organic acids, a sorbent based on celeolite, and an aromatase inhibitor helps to normalise the level of individual micro- and macronutrients and restore the antioxidant protection of the cow's body.

The use of the proposed treatment regimen increases the fertilising capacity of cows of the song

of the 1st calving by 18.15%, cows aged 3-4 years – by 30.12%, and cows of the older age group – by 40.47%, respectively. Further research on this subject should be aimed at a deeper understanding of the relationship between mycotoxicosis and the reproductive function of cows, in particular, with regard to molecular and hormonal mechanisms of influence. It is important to examine the effect of different types of mycotoxins on different

stages of the reproductive cycle and explore the possibilities of optimising complex drugs to improve heat.

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CONFLICT OF INTEREST

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Показники відтворення при використанні комплексного застосування препаратів за спонтанного прояву охоти у корів за мікотоксикозу

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Анотація. Все більшого значення набуває аліментарна неплідність зумовлена токсичним впливом мікотоксинів, особливо зearаленоном та деоксиніваленолом, що призводить до зниження ефективності діяльності галузі скотарства, тому необхідним є вивчення даної проблеми. Метою роботи було виявити вплив комплексного застосування сорбентів, підкислювачів та інгібіторів ароматази на відтворну здатність корів за мікотоксикозу. При проведенні досліджень було використано клінічні методи (загальне обстеження) біохімічні (вміст в сироватці крові кальцію, магнію, сечовини, загального та прямого білірубину, малонового діальдегіду, активність амінотрансфераз. Корів було поділено на дві групи: перша група була контрольною, де лікування

не застосовували, коровам другої групи застосовували комплексний метод лікування. Встановлено зниження рівня кальцію до $1,68 \pm 0,53$ ммоль/л, фосфору – $0,98 \pm 0,16$, селену – $0,64 \pm 0,1$ ммоль/л, підвищення рівня магнію до $1,15 \pm 0,17$ ммоль/л, підвищення аспартат амінотрансферази до $121,33 \pm 3,91$ Од, алатамінотрансферази до $79,31 \pm 6,53$ Од, загального білірубину до $10,21 \pm 0,55$ мкмоль/л, малонового альдегіду до $8,27 \pm 0,41$ мкмоль/л та Церулоплазміну до мкмоль/л $2,14 \pm 0,39$ при хронічному мікотоксикозі, зумовленого зеараленоном та деоксиніваленолом. При застосуванні комплексної схеми лікування тварин встановлено достовірну зміну та наближення до реферативних рівнів показників селену, креатиніні, АСТ, АЛТ, малонового альдегіду та церулоплазміну. Застосування запропонованої схеми лікування підвищує запліднюючу здатність корів після 1-го отелу на 18,15 %, корів віком 3-4 роки – 30,12 % та корів старшої вікової групи на 40,47 %. Запропоновано комплексний метод лікування корів за мікотоксикозу, зумовленого зеараленоном з використанням підкислювача на основі органічних кислот, сорбенту на основі целюліту та інгібітору ароматази, яка може бути використана в тваринницьких господарствах з виробництва молока та м'яса різної форми власності для відновлення та нормалізації відтворювальної здатності корів

Ключові слова: мікотоксини; відтворення; інгібітор ароматази; антиоксидантний захист