Qubahan Academic Journal
گوڤارا قبهان یا ئەکادیمی
مجلة قبهان الأكاديمية
Doi: 10.58429/Issn.2709-8206

Effect of Mycotoxins on Reproduction and Milk Quality of Dairy Cattle and its Amelioration by Natural Adaptogen "Betulin"

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https://doi.org/10.58429/qaj.v3n4a231

Abstract

The purpose of this study was to ascertain the effect of mycotoxins on the reproduction and milk quality and its amelioration by "Betulin" in lactating Holsteinized Russian Black Pied cows divided into 2 complexes which were further split into 2 groups. On general clinical examination of the reproductive tract, it was observed that the high milk yielding cows have low rates (82.14 ± 1.59%) of productive offspring in the extensive method, while it was higher (85.12 ± 2.31%) in the intensive method of rearing. During the experiment, mycotoxin contamination was observed in the feed, especially for Zearalenone (ZEN), the concentration of which was 9.5% in corn silage and 8.9% in concentrate feed. In cows, the presence of ZEN or Fusarium toxin contributed significantly to infertility and decreased milk production (8523.9±112.5 vs 7153.8±99.7 kg). However, lactating cows showed tolerance to mycotoxins, as evidenced by insignificant changes in the indicators of milk quality in subject animals. Further, the use of the natural adaptogen "Betulin" added to an increase in milk yield and an improvement in the quality and sanitary parameters of milk.

Keywords: cows, mycotoxins, quality indicators, milk, drug "Betulin".

1. Introduction

In concurrence with the current trend of intensifying dairy cattle rearing systems aimed at maximizing production and profits, new standards for feeding highly productive animals are being developed using various feed additives and supplements (Korotkiy et al. 2023; Avdeenko et al. 2021; Sherimova et al. 2022; Fedotov et al. 2020). However, in large livestock enterprises, nutritional infertility is diagnosed annually in 25-30% of infertile cows, indicating that this problem is quite widespread in dairy cattle breeding (Gnezdilova et al. 2021; Yakhaev et al. 2020). The most prevalent type of impeded fertility in dairy cows is nutritional infertility, which is brought on by changes in the energy content of diets, including changes in the amounts of proteins, carbohydrates, fats, macro and microelements, improper diet formulation and also the use of poor-quality feeds (Simonov et al. 2011).

In this context, emphasis should be placed towards both natural and undesirable contaminants, including mycotoxins, while examining the quality of feed used to feed lactating cows, which continue to have detrimental effects on both animal and human health (Coffey et al. 2009). Mycotoxins especially Fusarium species are commonly associated with contamination of grain (Glenn, 2007), which can occur prior to harvest in the crop fields or after it during storage or in silo trenches. The degree of poisoning is dependant on the techniques and storage conditions used and also on the geographical and climatic regions, temperature, humidity, aeration, infestations of insects, and physical damage to the cereals or grains (Placinta et al. 1999). According to previous research studies carried out, it was inferred that the Fusarium rot and mycotoxin contamination are highly prevalent during the rainy season due to significant temperature changes and also forage crops' considerable structural changes during this season. Due to carryover effects, mycotoxin residues can be found in animal products viz., eggs, milk, meat and offal and poses a potential risk to human health (Cavret&Lecoeur, 2006; Yiannikouris&Jouany, 2002).

Analysis of biological samples for mycotoxins enables evaluation of consumer risk associated with consumption of contaminated food products of animal origin as well as the degree of exposure to these toxins (Dänicke&Winkler, 2015). Additionally, it is well recognised that cow's milk, which includes almost all the vital nutrients essential for life is a crucial part of the diet of people of all ages. First and the foremost concern arising with milk are the kids who drink milk as one of their main food sources during their first few years of life. The goal of the current experiment was to ascertain how natural mycotoxins affected the health, pregnancy rate, milk yield, and milk quality characteristics of dairy cows. The level of mycotoxins in feed at a particular farm was determined using analytical techniques, and it was investigated whether enhancing feed with the chemical “Betulin” had a beneficial effect on dairy cattle. The goal of the study was to establish the impact of mycotoxins on production and propagative functions of cows reared under intensified production systems.

2. MATERIALS AND METHODS

The experiment was carried out on Holsteinized Russian Black Pied cattle during the winter- spring seasons of 2023 at the “Lednevo” breeding farm OJSC, Vladimirov region. The cows were reared under extensive and intensive livestock farming systems designated as complex 1 and complex 2 respectively. Holsteinized Russian Black Pied cattle with a live weight of 500.0 ± 5.0 kg were chosen for the experiment. These cattle were split into 2 complexes, 1 and 2, which were then divided into 2 groups by 20 animals. The first groups of both complexes 1 and 2 were control groups, wherein the animals received the basic control diet, while the cows of the second groups received the main diet along with the drug “Betulin” 300 mg per head for 21 days. The feeding and housing conditions for the control and the experimental animals were the same. The cows were fed standard cattle diets accepted on the farms, taking into account milk production, live weight and the physiological state of the animals. The diet for lactating cows on experimental farm during the winter period consisted of haylage, silage and mixed feed with the addition of pulp and cake as required by production, as well as meal and molasses.

Analytical and production experiments were carried out simultaneously at complex No.1 of JSC “Lednevo”, where cows were kept in tethers, with a productivity of 7000-7500 kg, and also at complex No.2 with free-stall housing with cow productivity of 9500 kg per lactation. During the experiment, the influence of natural adaptogen “Betulin” was also determined on the physiological and production levels of cows.

Mycotoxin concentrations in the study were measured using Ridascreen competitive ELISA kits for DON (Art. No. R5906), Zearalenone (Art. No. R1401), and Fumonisin (covering Fumonisin B1, B2, and B3) (Art. No. R3401), provided by R-Biopharm, Darmstadt, Germany. The analysis strictly adhered to the manufacturer's test protocols. The ELISA kits consisted of a 96-well microplate with antibody coating, standards with varying mycotoxin concentrations, enzyme conjugate, anti-antibody, and a chromogen solution (urea peroxide/tetramethylbenzidine). They also included a stop solution, along with buffers for washing and dilution. All additional chemicals used were of analytical grade. The ELISA tests were conducted on a ChemWell analyzer (Awareness Technology Inc. 2910, USA), measuring absorption at 450 nm. To assess the mycotoxin levels in milk samples, a separate standard curve was established as an illustrative reference using skimmed milk for each mycotoxin assay. The final mycotoxin concentration in the sample was determined by taking into account both the dilution factor and the average recovery rate calculated for each mycotoxin.

The detection (LOD) and quantification limits (LOQ) for Fusarium Mycotoxins were determined using ten commercially produced milk samples that were initially confirmed as toxin-free. These samples served as blank matrices. The LOD was calculated as the average value plus three times the standard deviation (LOD = mean ± 3SD), and the LOQ was defined as the average value plus six times the standard deviation (LOQ = mean ± 6SD). Mycotoxin recovery rates were tested at two concentrations: 10 and 50 mcg/l for DON and ZEN, and 50 and 100 mcg/l for FUM. For this assessment, toxin-free milk samples were spiked with a high concentration (200 mcg/l) of the respective mycotoxins. Three replicates at each concentration level were analyzed to ascertain the "peak" levels.

To conduct biochemical studies of blood serum, automatic biochemical analyzer BioSystems A25 (USA) was used. The physicochemical parameters of the milk of the tested cows were determined by classical methods approved by the current regulatory documentation and methodological recommendation (Fedotov et al. 2022). Milk sampling was carried out proportionally to milk yield using a graduated pipette, metered syringes, a measuring cup and numbered cups for transporting milk samples according to GOST R ISO 707-2010.

The assessment of the state of indicators of the reproductive ability of black-and-white cows was carried out by a detailed analysis of the data of the breeding records of the Selex program and also by thorough gynecological and obstetrical studies (Fedotov et al. 2018).

3. RESULTS

The general gynecological and obstetrical examination of the livestock of cows kept at 2 AOA complexes "Lednevo" revealed that highly productive cows have quite low rates of productive offspring i.e., 82.14 ± 1.59% in cows kept using the extensive method (complex No. 1), and 85.12 ± 2.31% in the intensive method. It was also noted that the length of service period was increased when compared to the general norm for Holsteinized Russian Black Pied cattle and amounted to 124.5±10.30; 126.29±11.41 and 120.75±11.69 days, respectively (Table 1).

Table 1. Results of the Analysis of the Reproductive Ability of Cows and Heifers in the Experimental Farm of JSC "Lednevo" Vladimir Region

Parameters	Complex No. 1 (intensive method)	Complex No. 1 (intensive method)
Calves obtained from cows, %	82.14±1.59	85.12±2.31
Calves obtained from heifers, %	93.14±2.22	92.21±1.14
Loss of calves due to abortions in cows, incl. embryonic mortality, %	16.64±1.27	10.42±0.98
Loss of calves due to abortions in heifers, incl. embryonic mortality, %	4.83±0.96	6.46±1.54
Service period, for cows, days	126.29±11.41	120.75±11.69
Infertility period of 1 forage cow, days	105,23±9.85	92.74±10.24

When analyzing feed rations, it was found that cows received 5.8-6.7 feed units daily on experimental farms. On the whole, the cows received less than 70 g of digestible protein, 14 g of phosphorus, 30 mg of carotene, while calcium was 24 g, in accordance with recommended standards for Holsteinized Russian Black Pied cattle.

Haylage and silage in the experimental farms were harvested on their own in concrete trenches and towers using preservatives and biologically active substances. At the same time, roughage was mainly intended for pregnant and lactating animals at the rate of 14 kg per cow per day. Dry matter consumption in the 1st month of lactation at JSC "Lednevo" averaged 18-20 kg.

The Vladimir region belongs to the zone of risky agriculture. The analytical data obtained gives an idea to assume the possibility of the influence of mycotoxins on the main feed crops. And as a consequence, the occurrence of nutritional infertility in the breeding stock of cattle due to consumption of poor-quality feed.

Table 2. Average Mycotoxin Content in Silage and Concentrates

Mycotoxins	Feed	Positive samples, %	Average values, μg/kg
DON deoxynivalenol	Silage	81.9	3.901±0.093
	Concentrates	72.1	2.152±0.104
ZEN zearalenone	Silage	73.8	2.093±0.088
	Concentrates	57.7	526.141±14.138
FUM fumonisins	Silage	88.1	849.004±9.116
	Concentrates	70.7	854.912±11.431

During the research, lactating cows were fed with feed made from cereals grown in year 2022. Concentrations of mycotoxins in feed selected from the dairy farms of OJSC "Lednevo" in 2023 are presented in Table 2.

In total, DON (deoxynivalenol) was detected in 77%, ZEN (zearalenone) in 66%, and FUM (fumonisins) in 80% of corn silage and concentrate samples. The concentrations of DON and ZEN exceeded the recommended levels for feeding dairy cattle. While FUM concentrations did not surpass the recommended thresholds in any of the samples, there were notable levels of contamination present. During the experiment, a high level of feed contamination was observed, especially with ZEN, the concentration of which was high: 9.5% of the recommended samples were corn silage and 8.9% of concentrated feed samples.

The significant levels of mycotoxin contamination we observed can be attributed to climatic conditions, particularly high humidity and temperature, which are critical factors for mold formation. These weather conditions during the growth and harvesting of forage crops likely contributed to the elevated concentrations of mycotoxins in the feed.

Due to the high concentration of mycotoxins in feed and a slight excess of the norm in milk, the present experimental work was directed towards determination of influence of the natural adaptogen "Betulin" on the milk productivity and also the quality indicators of cows' milk.

Table 3. Average Mycotoxin Content in Cow's Milk

Mycotoxins	LOD (μg/L)	LOQ (μg/L)	Positive samples, %	Average values, μg/kg
DON deoxynivalenol	4.0	5.4	14.3	21.122 ±3,814

ZEN zearalenone	0.8	1.2	94.3	5.503±1.446
FUM fumonisins	20.1	25.3	92.4	77.319±14.285

The research included quality control of raw milk in accordance with GOST R 52054-2003 and Technical Regulations for Milk and Dairy Products from July 22, 2010, No. 163 - Federal Law. Organoleptic, physicochemical, sanitary and hygienic indicators of milk were analyzed according to generally accepted methods. The milk yield of cows of the first group of cows kept in complex No. 2 (intensive method of housing) of JSC "Lednevo" for 305 days of lactation, exceeded the milk yield of cows of the second group by 13.1% (8523.9 ± 112 .5 versus 7153.8±99.7 kg); in cows from complex No. 1 (extensive method of housing), the milk productivity of group I was significantly higher (6917.3±95.9 versus 5588.7±101.6 kg) as shown in Table 4. The yield of dry matter (DM), milk fat (MF), milk protein (MP) and lactose (L) in animals of 1st group, both with intensive and extensive methods of housing, was also higher than in animals of 2nd group. Accordingly, in cows at complex No. 1, it was 782.4 ± 4.6 vs 574.6 ± 4.5 kg (DM); 243.9±8.2 vs 173.1±5.1 (MF); 201.9±9.2 vs 188.1±5.2 (MP) and 301.1±4.7 vs 227.2±8.3 (L). Similar dynamics in milk were observed in cows kept on complex No. 2 as well (intensive method) (Table 4).

The most valuable component in the composition of milk is dry matter, which is based on milk fat, milk protein, milk sugar and minerals, vitamins, enzymes, hormones, immune bodies and pigments (Fedotov et al. 2022). According to the present study, in the first group of experimental cows, the amount of dry matter on complex No. 2 was 943.4 ± 4.6 kg; while it was 782.4 ± 4.6 kg in the extensive complex 1.

Table 4. Milk Productivity of Cows for 305 Days of Lactation at JSC "Lednevo" (n=20)

Parameters	Complex No. 2 (intensive method)		Complex No. 1 (extensive method)	
	Group I	Group II	Group I	Group II
Milk yield for 305 days of lactation, kg	8523.9±112.5	7153.8±99.7*	6917.3±95.9	5588.7±101.6*
Dry matter yield, kg	943.4±4.6	771.53±8.5*	782.4±4.6	574.6±4.5*
Milk fat yield, kg	454.0±9.2	384.5±7.7*	243.9±8.2	173.1 ±5.1*
Milk protein yield, kg	391.0±7.4	321.7±5.4*	201.9±9.2	188.1±5.2*
Lactose yield, kg	481.3±6.7	422.6±9.1*	301.1±4.7	227.2±8.3*

* – P < 0.05. ** – P < 0.01. *** – P < 0.001.

An important consideration during the production of cottage cheese and other milk products is not only the content of total protein, but also its other components: casein and whey proteins, the content of which determines the rennet, coagulability and yield of the finished product (Fedotov et al. 2019; Zhanadayev et al. 2022; Nasiyev&Bekkaliyev, 2019). The total protein content in group I of cows of complex No. 2 was 3.17±0.10%, and in group II it changed slightly and amounted to 3.15±0.12%, however, the casein content in group 2 decreased by 5.9% and amounted to 2.57±0.16%. In turn, the content of serum proteins increased in group 2 and amounted to 0.69±0.10% albumin.

In the studies involving milk from cows in control groups, we observed a decrease in the dry matter content and alterations in the quantitative composition of milk components. Table 5 indicates that the dry matter content in the milk from cows in the first experimental group was consistently higher than that in the second group across all complexes. Furthermore, when milk obtained from sick cows was subjected to heating, we observed the destabilization of certain whey proteins. The transition of destabilized whey proteins from a dissolved state to an insoluble state was accompanied by their precipitation. This is due to the fact that excess whey proteins are deposited on the walls of the heating unit. This condition of milk does not allow it to be used to produce quality milk products.

Table 5. Qualitative Composition of Milk from Cows in JSC “Lednevo” (n=20)

Parameters	Complex No. 2 (intensive method)		Complex No. 1 (extensive method)	
	Group I	Group II	Group I	Group II
	±	±	±	±
Dry matter content in milk, %	13.14±0.22	10.88±0.25 *	14.7±0.14	11.8±0.12*
Dry matter including SOMO, %	8.34±0.33	7.54±0.24	8.22±0.19	7.91±0.14*
Fat, %:	3.79±0.18	2.89±0.12	4.19±0.14	3.31±0.18*
Total protein, %:	3.17±0.10	3.15±0.12	3.15±0.11	3.09±0.22
Casein, %:	2.74±0.23	2.57±0.16	2.54±0.09	2.18±0.18
Albumins, %	0.53±0.17	0.69±0.10	0.41±0.02	0.52±0.02
Globulins, %	0.58±0.10	0.19±0.08	0.42±0.04	0.22±0.01*
Lactose, %	4.89±0.17	4.34±1.17	4.61±0.17	4.24±0.15

* – P < 0.05. ** – P < 0.01. *** – P < 0.001.

The titratable acidity of milk of subject cows of the first group of JSC “Lednevo” was within the limits provided for by GOST, for the prepared milk, while it was reduced in cows of the second group from 13.65±0.24 to 13.17±0.26°T, which indicates an inflammatory process in the mammary gland (Table 6). This conclusion is confirmed by the content of somatic cells, which was higher in animals of group II compared to group I, which also indicates the onset of the inflammatory process in the mammary gland. The density of milk of cows of all groups corresponded to the norm stipulated by GOST, but in cows of the second experimental group (extensive method of rearing) it was at the lower limit and amounted to 1026.11±1.51 kg/m3. In cows kept under the intensive method, the number of somatic cells in the milk of the second group significantly increased (4.54·105±0.29×105 vs 3.28·105±0.19×105), and accordingly with the extensive method, 4 .33×105 ±0.24×105 vs

3.25×105±0.19×105 in cm3. On the whole, the use of the natural adaptogen “Betulin” contributed to an increase in milk yields and improved quality and sanitary-hygienic indicators of milk.

Table 6. Sanitary and Hygienic Indicators of Milk from Cows in JSC “Lednevo” (n=20)

Parameters	Complex No. 2 (intensive method)		Complex No. 1 (extensive method)	
	Group I	Group II	Group I	Group II
	±	±	±	±
Acidity, °T	16.83±0.31	13.64±0.29*	15.94±0.57	13.17±0.26*
Density, kg/m3, not less	1027.93±4.83	1027.77±2.11	1027.96±1.37	1026.11±1.51
The content of somatic cells, cm3, not more	3.28×105±0.19×105	8.54×105±0.29×105	3.25×105±0.19×105	4.33×105±0.24×105

* – P < 0.05. ** – P < 0.01. *** – P < 0.001.

4. CONCLUSIONS

In cows, the presence of zearalenone or Fusarium species producing this mycotoxin contributed to infertility and decreased milk production (8523.9±112.5 vs. 7153.8±99.7 kg). However, lactating cows showed tolerance to mycotoxins, as evidenced by insignificant changes in the quality indicators of milk in animals of the control groups. Our hypothesis suggests that in ruminants, the rumen microflora may potentially transform several mycotoxins into metabolites that pose minimal or no health risks. This natural defense mechanism in healthy animals serves as a crucial barrier against various ruminant diseases. In contrast to monogastric animals like pigs, ruminants typically exhibit greater resistance to the detrimental effects of mycotoxins. Further, the use of the natural adaptogen “Betulin” contributed to an increase in milk yield and an improvement in the quality and sanitary-hygienic indicators of milk. These results can serve as a solid foundation for further research.

ACKNOWLEDGMENTS

The present research was taken up by the employees of Department of Disease Diagnostics, Therapy, Obstetrics and Animal Reproduction Gnezdilova L.A., Fedotov S.V., Muradyan Zh.Yu., Rozinsky S.M. within the framework of the Russian Science Foundation grant (agreement No. 23-26-00150).

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