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Mycotoxin risk management for dairy cows by monitoring blood parameters, reproduction status and SCC in milk

[*Gerenciamento de risco de micotoxinas para vacas leiteiras através do monitoramento de parâmetros sanguíneos, status de reprodução e SCC no leite*]

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ABSTRACT

The objective of this study was to determine the effectiveness of mycotoxin management with feed additive by monitoring biochemical blood parameters, reproduction status and udder health in cows. During the first 1-12-months, the reproduction performance was assessed. The cows were fed only total mixed ration (TMR) with naturally contaminated mycotoxins (ZEN;DON;AFB_1;OTA) and the cows were regarded as a control group (CG). In months 13-15, two groups were created: control (CG)(n=30) and experimental (EG)(n=60). The CG was fed with contaminated TMR and the EG was fed with the same TMR+40g/cow mycotoxins management feed additive (TMXL1000). During this period, blood indicators and udder health were studied. Beginning with months 16-24, all cows were fed with contaminated TMR+40 g/cow (TMXL1000) and regarded as the EG. The IgA concentrations in the CG decreased in the 15th month ($p<0.05$). The concentrations of cortisol decreased by two times ($p<0.05$) in the EG. Ovarian cyst treatment was more effective by 14.98% ($p<0.05$) in the EG than in the CG ($p<0.05$). The EG performed an effective (18.02%) (*$p<0.05$*) response to applied ovsynch protocol compared with the CG. According to the obtained results, it can be concluded that feed additive for mycotoxins management had a positive impact on dairy cow health.

Keywords: dairy cows, feed additive, health, mycotoxins, reproduction

RESUMO

O objetivo deste estudo era determinar a eficácia do manejo de micotoxinas com aditivo alimentar através do monitoramento de parâmetros bioquímicos do sangue, estado de reprodução e saúde do úbere em vacas. Durante os primeiros 1-12 meses, o desempenho de reprodução foi avaliado. As vacas foram alimentadas apenas com ração total mista (TMR) com micotoxinas naturalmente contaminadas (ZEN;DON;AFB1;OTA) e as vacas foram consideradas como um grupo de controle (CG). Nos meses 13-15, dois grupos foram criados: controle (CG)(n=30) e experimental (EG)(n=60). O CG foi alimentado com TMR contaminado e o EG foi alimentado com o mesmo TMR+40g/ aditivo para o manejo de micotoxinas de vacas (TMXL1000). Durante este período, foram estudados os indicadores de sangue e a saúde do úbere. A partir dos meses 16-24, todas as vacas foram alimentadas com TMR+40 g/ vaca contaminada (TMXL1000) e consideradas como o EG. As concentrações de IgA no GC diminuíram no 15º mês ($p<0,05$). As concentrações de cortisol diminuíram duas vezes ($p<0,05$) no GE. O tratamento do cisto ovariano foi mais eficaz em 14,98% ($p<0,05$) no GE do que no GC ($p<0,05$). O GE realizou uma resposta efetiva (18,02%) ($p<0,05$) ao protocolo ovsynch aplicado em comparação com o GC. De acordo com os resultados obtidos, pode-se concluir que o aditivo alimentar para o manejo de micotoxinas teve um impacto positivo na saúde das vacas leiteiras.

Palavras-chave: vacas leiteiras, aditivo alimentar, saúde, micotoxinas, reprodução

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INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi that grow naturally in several agricultural commodities, causing a wide range of toxic effects in vertebrates (Adetunji *et al.*, 2014). Mycotoxins are produced by certain fungal species (*Aspergillus*, *Penicillium*, *Fusarium* spp.). *Fusarium* genus is a frequently found contaminant in feed and feed materials. Feed materials can be contaminated with mycotoxins in two ways: during the growth period in field or during the storage period.

Several mycotoxins are produced by more than one type of microscopic fungi. The research revealed that most commonly, feed material and feed is contaminated by 2 to 4 different types of mycotoxins. It was also determined that Lithuania's climate is notably favorable for *Fusarium* fungi spread (Adetunji *et al.*, 2014; Mankevičienė *et al.*, 2014).

Mycotoxins can affect animal health and their products to varying degrees. Also, mycotoxins cause a significant decrease in dairy cattle productivity and reproduction function, this has also been shown by our studies (Elliott *et al*., 2020; Rodriguez-Blanco *et al.*, 2020).

Dairy cows may be regularly exposed to a mixture of mycotoxins derived from different ingredients in their diets. Other studies focused on investigating basic blood biochemical parameters and animal performance suggest that dairy cows are more unresponsive to mycotoxins than monogastric livestock because mycotoxins are readily degraded by rumen microbes. However, mycotoxins have a negative impact on most organ systems of dairy cows (Santos and Fink-Gremmels, 2014).

AFB_1 is characterized as a strong hepatotoxin and hepatocarcinogen. AFB_1 most commonly influences the reproductive and immune systems. Liver is the main target of this mycotoxin. Feed contaminated with ZEN can cause reproduction and endocrine system disorders, genitalia oedema and hypertrophy in cows, high embryonic mortality in in-calf cows, decreased luteinized hormone (LH) and progesterone rates (which can affect the morphology of uterus tissue), and reduced milk production. Also, oxidative stress is evoked because of DNA disturbance. Also, ZEN is a non-steroid estrogenic mycotoxin that has an impact on cattle reproductive tract malfunctions (Liu *et al.*, 2019). DON inhibits protein synthesis, therefore mostly affected are cells actively producing proteins – lymphocytes and liver. In liver predominantly malfunctions the synthesis of albumin and fibrinogen. When organism is exposed to deoxynivalenol there is an increase in cytokine and therefore immune system is suppressed and apoptosis mediator caspase-3 is activated. Scientists have revealed that mycotoxins can affect organisms in different ways. Deoxynivalenol and T-2 toxin affects digestive tract and reproductive system; fumonisins – nervous system and lungs; ochratoxin A – liver, kidneys, digestive tract, and immune system (Nidhi and Manisha, 2013). Ochratoxins (OTA) effects of on animal health are strongly influenced by its kinetic toxicokinetic parameters. In the bloodstream, almost all OTA is strongly bound to serum proteins, mainly albumin (Battacone *et al.*, 2010).

Mycotoxins can also cause stress in the organism. Stress is one of the factors that have an impact on cattle health. Stress hormone cortisol is the essential criterion determining the stress level in animal. Cortisol is produced when hypo-thalamic-pituitary-adrenal (HPA) axis is activated during a stressful situation (Burnett *et al.*, 2014).

The negative energy balance (NEB) in dairy cattle is directly related to the suppression of the immune system. To avoid it, feed materials must be protected from possible contamination with fungi secondary metabolites. When contaminated is unavoidable, the usage of detoxifying substances is necessary. The aforementioned group is also known as adsorbents or binders which decrease mycotoxin passage to blood and target organs after accessing cow's organism. During this study, we used a bio transformative substance which dissolve mycotoxins into less toxic metabolites (Döll and Dänicke, 2011; Wambacq *et al*., 2016).

The aim of this study was to determine the effectiveness of mycotoxins management with feed additive regarding biochemical blood parameters, reproduction status and udder health in dairy cows.

MATERIALS AND METHODS

Ninety Lithuanian Black and White dairy cows were selected according to the following criteria: 30 days *postpartum*, in their second or higher lactation (on average 3.0 ± 0.45 lactations), without any clinical symptoms of ketosis, acidosis, mastitis, metritis, or other diseases. The study aimed at cows' reproduction performance assessment lasted for 24 months. During the first 12 months, cows was fed total mixed ration (TMR) with naturally contaminated mycotoxins, without feed additive for mycotoxins managing. These cows were regarded as control group (CG). On the 13^{th} to 15^{th} months the dairy cows were divided into two groups: control (CG) (n=30) and experimental (EG) (n=60). The cows of CG were fed same TMR with naturally contaminated mycotoxins, meanwhile dairy cows of EG were fed contaminated TMR plus 40 g/cow mycotoxins managing feed additive. In this period (from 13 to 15 months) we investigated the blood indicators and udder health. From 16^{th} to 24^{th} months, all dairy cows were fed contaminated TMR plus 40g/cow mycotoxins managing feed additive and were regarded as EG group. TMR components are formulated accordingly to meet or exceed the requirements of a 500kg Lithuanian Black and White dairy cows producing 28±0.5kg/day of milk. TMR for cows was composed of 45% grass silage, 20% corn silage, 15% grass hay, 15% grain concentrate mash (50% barely and 50% wheat) and 5% of mineral additive (main components: calcium, phosphorus, and zinc hydroxychloride). The ration was composed of dry matter (%) – 49.2; neutral detergent fiber (% of DM) 26.2; Crude protein (% of DM) – 16.2; Crude ash (% of DM) – 19.6; Net energy for lactation (Mcal/kg) - 1.4. Mycotoxin management feed additive (TMXL 1000, TechMix Europe, Spain) the main component are wall polysaccharides from yeasts (*Saccharomyces cerevisiae*), dried culture of *Aspergillus oryzae* in flour of barley, and dried extract of fermentation *Kluyveromyces marxianus*. The analytical constituents of TMXL 1000: magnesium 2.5%, sodium 1%, calcium 0.5%, lysine 0.5%, methionine 0.5%. The chemical composition of TMRs samples after drying were determined by laboratory analysis with NIR spectrometer (Foss InfraXact NIR analyzer). Cows were housed in a ventilated tie-stall barn on stall (180 × 130 cm) and were allowed to go outside for exercise two times per day. Water consumption was not limited (*ad libitum*). The cows were fed throughout the year at a certain fixed time, at 07:00 p.m. (GTM+2) and 07:00 a.m. (GTM+2).

The permit for study of test animals was received from the Lithuania's State Food and Veterinary Service (SFVS) (permit No. G2-102).

The concentrations of mycotoxins aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), deoxynivalenol (DON), T-2 toxin, (T-2), HT-2 toxin (HT-2), zearalenone (ZEN), ochratoxin A (OTA) in TMR samples were tested by high-performance liquid chromatography (HPLC) with MS/MS detection or fluorescent detector (FLD) and Ultraviolet detector (UVD) described by CEN/TC 327 for ZEN, CEN/TC 275 for OTA, for the DON following the method of MacDonald *et al*. (2005) and T-2, HT-2 R-BIOPHARM RHÔNE LTD instructions. A total of 1kg mixed ration feed samples were collected 24 times (at the beginning of each study month) from different feeding places and stored in the dark at -20°C until the date of analysis. Feed samples were dried at 70^{o}C for 24h in a ventilated oven, 100g feed sample was ground to pass a 1mm screen and then stored for subsequent analysis. Samples of 5g were used for mycotoxin analysis.

Briefly, AFB_1, AFB_2, AFG_1 and AFG_2 were extracted using 20mL of MeOH (methanol)/H_2O (deionized water) (80/20, v/v) for 2h in an orbital shaker RS-OS 20 (Germany) and purified using an immuno-affinity column AFLAOCHRA PREP® (R-Biopharm, Germany). The ZEN was extracted using 25mL of ACN (Acetonitrile)/H_2O (75/15 v/v), containing NaCl (sodium chloride) (5g per 100mL) for 2h in an orbital shaker and purified using an immuno-affinity column EASI-EXTRACT® ZEARALENONE (R-Biopharm, Germany). The OTA was extracted with 20 ml of ACN/H_2O (65/35, v/v) for 2h in an orbital shaker and purified using an immuno-affinity column AFLAOCHRA PREP® (R-Biopharm, Germany). The T-2 and HT-2 were extracted using 25mL of MeOH/H_2O (90/10) containing NaCl (5g per 100mL) for 2h in an orbital shaker and purified using an immuno-affinity column EASI-EXTRACT® T-2 & HT-2 (R-Biopharm, Germany). AFB_1, ZEN, OTA, T-2 and HT-2

were analyzed by HPLC-FLD (Model LCMS-8060 Shimadzu Corporation, Kyoto, Japan). Chromatographic conditions for AFB_1 were HPLC column: 250-4 RP-18e (5µm), fluorescent detector: PMT Gain 4, mobile faze: H_2O/ACN/MeOH (6/2/3, v/v), column temperature: 30°C, injection volume: 20µL. Method limit of detection - 0.01µg/kg. Chromatographic conditions for ZEN: HPLC column: 250-4 RP-18e (5µm), fluorescent detector: PMT Gain 10, mobile phase: H_2O/ACN/MeOH (46/46/8, v/v/v), column temperature: 30°C, injection volume: 100µl. Method limit of detection - 3µg/kg. Chromatographic conditions for OTA: HPLC column: 250-4 RP-18e (5µm), fluorescent detector: PMT Gain 15, mobile faze: ACN/H_2O/HNO_3 (48/51/1, v/v/v), column temperature: 30°C, injection volume: 100µl. Method limit of detection - 0.01µg/kg. Chromatographic conditions for T-2 and HT-2: HPLC column: 250-4 RP-18e (5µm), fluorescent detector: PMT Gain 11, mobile phase: ACN/H_2O (50/50, v/v), column temperature: 40°C, injection volume: 100µl. Method limit of detection – 1.4µg/kg. The DON was extracted with 1g PEG (polyethylene glycol) and 40 mL of H_2O mixture for 1 h in an orbital shaker and purified using an immuno-affinity column DONPREP® (R-Biopharm, Germany) and analyzed by HPLC-MS (Model Sciex API 5000, McKinley Scientific, USA). Chromatographic conditions for DON: HPLC column: 250-4.6, RP-18-300 (5µm), mobile faze: H_2O/MeOH/ACN (94/3/3, v/v/v), column temperature: 30°C, injection volume: 100µL. Method limit of detection - 20µg/kg.

Blood samples were collected from 13th to 15th study month at 07:00 a.m. before feeding from tail venae (*v. caudalis*) into vacuum test tubes without additives (BD Vacutainer®, Great Britain). The collected blood samples were stored in an ice bath until all samples were taken. Biochemical blood samples were evaluated at National Food and Veterinary Risk Assessment Institute (NFVRAI) (Lithuania). Automatic biochemical analyzer "COBAS INTEGRA® 400 plus" (Tegimenta Ltd, Roche, Switzerland) was used. The samples were analyzed to estimate alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), creatinine (CREA), total bilirubin (BIL-T), glucose (GLUC), total protein (TB), gamma-glutamyl transferase (GGT), calcium (Ca), lactate dehydrogenase (LDH), magnesium (Mg) and urea (UREA) parameters. Immunoglobulin A (IgA) concentrations in blood serum samples was assessed using the Immunoperoxidase Assay for Determination of IgA in Bovine Samples kit, (GenWay Biotech, Inc., San Diego, CA). The absorbance was measured at a wavelength of 450nm with a spectrophotometer (Synergy HT, Bio – TEK, microplate reader, USA). Cortisol in blood serum samples was estimated using Bovine Cortisol ELISA Kit (Biomatik, United States), limit of detection: 0.049ng/mL – 200ng/mL. The cortisol concentrations also were measured at a wavelength of 450nm with a spectrophotometer. The reproduction status data was collected on the 24th months. The dairy cows were examined using ultrasound device CareSono HD 9300 Vet 6.5 MHz (Caresono Technology Co., Ltd) twice every 7 days according to their ovarian functionality. The dairy cows were stimulated using two different stimulation protocols (F2α and Ovsynch). Cows with 20 – 25mm diameter corpus luteum found in their ovaries received heat stimulation with prostaglandins. F2α protocol was prostaglandin stimulation PGF2 Enzaprost (France); 2 times per day 5 ml were injected with 12 to 24 hours interval. Cows were inseminated after visible heat characteristics after 3 or 3.5 days. If located *corpus luteum* and follicle diameter was less than 20mm Ovsynch protocol was practiced – on the 1st day 2.5mL GnRH (Ovarelin, France) were injected, on the 7th day 5mL PGF2α (Enzaprost, France) were injected and after 48 hours a repeated 2.5 mL GnRH injection was given; cow's insemination was performed 16 – 18 hours after the last treatment. The effectiveness of stimulation was measured by the level of pregnancy.

Milk samples were collected from the 13th to 15th study months. The somatic cell count (SCC) was evaluated by using Somascope (CA-3A4, Delta Instruments, the Netherlands), which operates on the principle of flow cytometry technology. The total bacterial count (TBC) was evaluated by using Bentley Bactocount IBC (Bentley Instruments Inc, USA) that uses flow cytometry for the rapid enumeration of individual bacteria in raw milk.

Statistical analysis was accomplished with software SPSS; version 25 (IBM Corp., USA) was used for the statistical analysis of the data. Blood parameters we analyzed in two ways, repeated analysis of variance considering effects on group, time and group by time interaction, and random effect of cow was used for mixed models' linear procedure. The Student's t-test and Fisher LSD (Least Significant Difference) test were used to determine the differences among the mean values of blood characteristics. The differences were considered statistically significant when $p<0.05$ otherwise declared as not significant.

RESULTS

The mycotoxins concentration in TMR samples on the 1^{st}-12^{th} study months were AFB_1 – 5.0±0.75µg/kg of DM, ZEN – 530.0±79µg/kg of DM, DON – 25.0±3.75µg/kg of DM, OTA – 15.0±2.25µg/kg of DM, on the 13^{th} study month: AFB_1 – 3.0±0.45µg/kg of DM, ZEN – 500.0±75µg/kg of DM, DON – 76.8.0±11.52µg/kg of DM, OTA – 20.6±3.09µg/kg of DM, on the 14^{th} study month: AFB_1 – 3.0±0.45µg/kg of DM, ZEN – 670.0±100µg/kg of DM, DON – 74.0±11µg/kg of DM, OTA – 8.8±1.32µg/kg of DM, on the 15^{th} study month: AFB_1 – 3.0±0.45µg/kg of DM, ZEN – 500.0±75µg/kg of DM, DON – 175.8±26.37µg/kg of DM, OTA – 28.3±4.2µg/kg of DM, and on the 16^{th}-24^{th} study months: AFB_1 – 1.7±0.26µg/kg of DM, ZEN – 550.0±82µg/kg of DM, DON – 164.0±24.6µg/kg of DM, OTA – 17.0±2.62µg/kg of DM. AFG2, AFB2, AFG1, T-2, HT-2 detected below the limit of detection in the TMR.

Statistically the significant parameters (*$p<0.05$; $p<0.001$*) and parameters whose concentrations did not meet reference limits during all study are given in Table 1. Other blood parameters during the study were detected within the reference limits. Parameters of CREA and GGT in the EG on the 13^{th} month was higher than the reference limits, on the 15^{th} month both parameters decreased respectively 43.13±0.01% *($p=0.003$)* and 20.63±0.01% (*$p=0.111$*). The concentration of Ca and Mg in blood samples on the 13^{th} month in EG were below reference limits, on the 15^{th} month respectively increased by 75.68±0.01% *($p<0.001$)* and 44.94±0.01% *($p=0.001$)*. Biochemical blood parameters except Ig A in CG cows were not affected and reached the references value during 13^{th}-15^{th} month study. IgA concentration decreased significantly in the CG on the 15^{th} month comparing with the 13^{th} month (Table 1). On the 13^{th} month, cortisol concentration was almost equal in both groups. But on the 15^{th} month, cortisol concentration decreased more than two times (*$p<0.01$)* in EG and increased 32.71±0.01% (*$p<0.05$)* in CG (Table 1).

In the present study were monitored ovaries functionality disorders (Figure 1A). The analysis showed that a by 6.4% (*$p>0.05$*) higher number of cows in the EG did not have changes in ovaries functionality compared with the CG. It was determined that in the CG ovaries hypofunction disorders were by 12.5% (*$p<0.05$*) more common but diagnosed cysts in cows' ovaries were by 6.1% (*$p<0.05$*) higher in the EG. It was determined that ovaries functionality disorders treatment in EC was by 14.98% (*$p<0.05$)* better than in the CG (*$p<0.05$)* (Figure 1B) and treatment of hypofunctioning ovaries was more efficient (1.92%) (*$p<0.05$)* in the CG than in the EG. The study demonstrated that the EG had an 18.02% *($p<0.05$)* better reaction in ovaries functionality to applied ovsynch protocol in contrast with the CG. F2α stimulation protocol did not receive any significant reaction in both groups *($p<0.05$)* (Figure 1C).

SCC and TBC can be used as indirect indicators of udder health. Total bacterial count data during the 13^{th}-15^{th} months study did not change in both groups so data are not shown. On the 13^{th} month of the study, SCC in both groups were approximately equal

and did not change significantly in CG during the 13^{th} - 15^{th} months study time, but in the EG this parameter decreased 55.37±0.01% ($p<0.05$) on the 15^{th} month study (Figure 2).

Table 1. The IgA, cortisol and biochemical blood parameters in dairy cows groups

Parameters	Months	Control group (CG)	Experimental group (EG)	RMSE	*p-value*			Reference limits
					Treatment	Time	T × T	
Biochemical blood parameters								
CREA, (µmol/L)	13 months	84	**123.8**	9.42	*0.02**	*0.001***	*0.03**	40-80[1]
	14 months	75	78.4		*0.65*			
	15 months	61.2	70.4		*0.13*			
	p-value (Time)	*0.003**	*0.001***	-	-			
	Overall	**73.4**	**90.87**	5.76	*0.02**			
GGT, (U/L)	13 months	29.3	**110.1**	38.33	*0.25*	*0.2*	*0.37*	17-51[1]
	14 months	29.44	36.84		*0.42*			
	15 months	36.92	54.34		*0.2*			
	p-value (Time)	*0.111*	*0.318*	-	-			
	Overall	**31.89**	**67.09**	22.49	*0.16*			
Ca, (mmol/L)	13 months	**2.04**	**0.62**	0.37	*0.05**	*0.001***	*0.03**	2.1-2.7[1]
	14 months	2.47	2.43		*0.69*			
	15 months	2.56	2.55		*0.96*			
	p-value (Time)	*0.25*	*0.001***	-	-			
	Overall	**2.35**	**1.87**	0.2	*0.04*			
Mg, (mmol/L)	13 months	0.93	**0.49**	0.13	*0.07*	*0.002**	*0.03**	0.85-1.2[1]
	14 months	1.11	1.21		*0.17*			
	15 months	0.9	0.89		*0.68*			
	p-value (Time)	*0.26*	*0.001***	-	-			
	Overall	**0.98**	**0.86**	0.06	*0.07*			
Ig A, (ng/ml)	13 months	406	392	51.6	*0.58*	*0.01**	*0.01**	-
	14 months	259	395		*0.59*			
	15 months	169	410		*0.38*			
	p-value (Time)	*0.01**	*0.37*	-	-			
	Overall	***278***	***399***	29.79	*0.001***			
Cortisol, (ng/ml)	13 months	12.42	12.48	4.8	*0.14*	*0.23*	*0.08*	
	14 months	22.52	11.3		*0.04**			
	15 months	18.46	5.92		*0.02**			
	p-value (Time)	*0.05**	*0.01**	-	-			
	Overall	**17.8**	**9.9**	2.77	*0.02**			

CREA, Creatinine; Ca, Calcium; GGT, Gamma-glutamyl transferase; Mg, Magnesium; IgA, immunoglobulin A; RMSE, root mean square deviation; *p<0,05; **p<0,001; T×T, two factors Time x Treatment relation influence, *p-value*; [1]Regulated norms set by BAYR MED LT 2000".

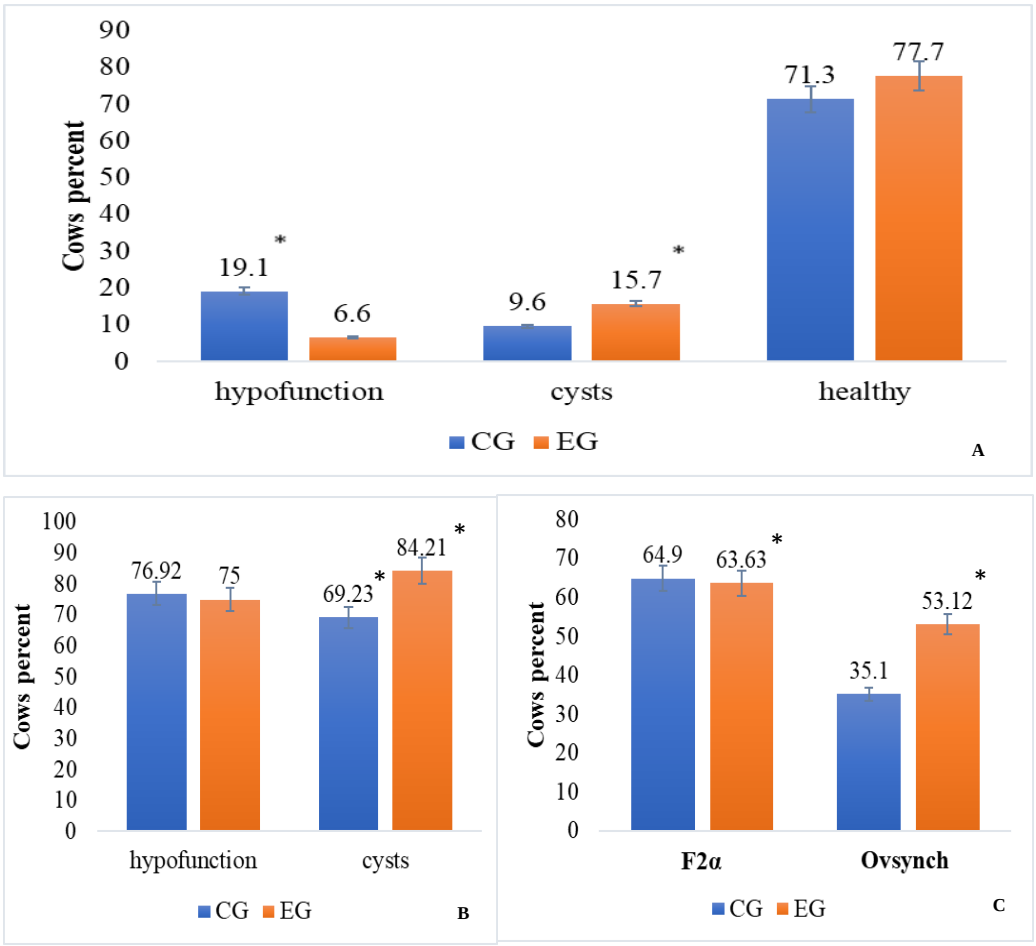

Figure 1 (**A)** Dependence of ovarian functional disorders in dairy cow groups on the 24th month of study (*p<0.05) (CG - dairy cows feeding TMR without feed additive for mycotoxins management; EG - dairy cows feeding TMR with feed additive for mycotoxins management), **(B)** – Recovery percent of dairy cows ovaries functionality disorders during the study (*p<0.05). (**C**) – Efficiency of hormonal stimulation protocols impact to ovaries functionality of dairy cows (*p<0.05). (CG - dairy cows feeding TMR without feed additive for mycotoxins management; EG - dairy cows feeding TMR with feed additive for mycotoxins managing).

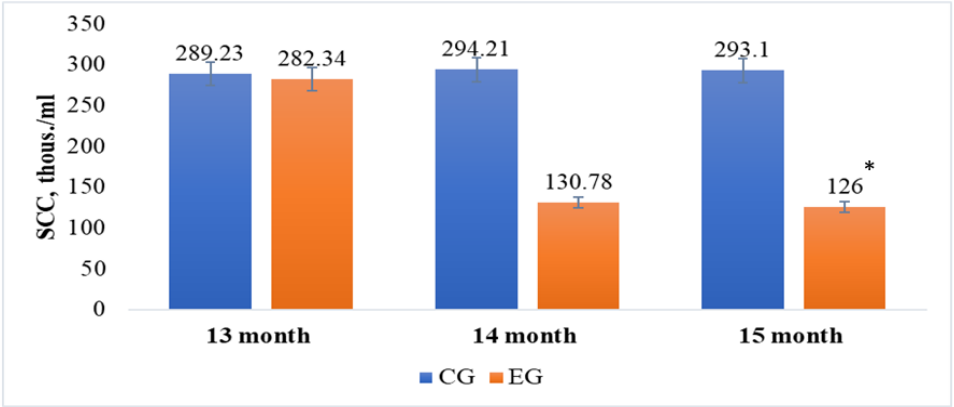

Figure 2. Somatic cell count at 13-15 month study in dairy cow groups (*p<0.05).

DISCUSSION

According to scientific literature, ruminants are less susceptible to the negative effect of mycotoxins than monogastric animals. The most notable difference between these types of animals is that mycotoxins can be degraded and/or bio converted in the rumen by local microorganisms (Wang *et al.*, 2019).

Creatine is a residual product formed from CREA located in organisms by spontaneous dehydration method. The increase in CREA concentration in blood samples hints renal malfunction, especially when glomerular filtration is decreased. In the present study, when dairy cows were fed the feed additive, CREA declined. It is believed that feed additive for mycotoxins management protects kidneys functionality. In the present study it was observed that the CREA parameter in the EG after two months feeding feed additive for mycotoxins managing was within the reference limits. The results of this study agree with those of Chinese researchers' who observed that after feed was contaminated with various concentrations of AFB_1 the biochemical blood parameters (including CREA) did not demonstrate significant variations (Wang *et al.*, 2019). The most common cause of dairy cows' health issues is a malfunction in calcium (Ca) metabolism, when calcium deficiency can affect nerve and muscle function. According to Antanaitis and Žilaitis (2016), the inadequacy of Ca has an influence on the cattle reproduction system and mastitis occurrence. Moreover, scientists suggest that an increase of Ca concentration in blood might expose sub-clinical mastitis. Before starting using feed additive for mycotoxins managing, in the EG the Ca concentration was twice as low as the reference limits. After two months of using feed additive for mycotoxins management, this parameter was within the reference limits. Also, this can be associated with appropriate mycotoxins risk management. However, Simion (2010) together with a group of scientists observed that feed contaminated with fungi and mycotoxins can disrupt the liver bile system, which may lead to hypercalcemia and hyperphosphatasemia (Simion *et al.*, 2010). The deficiency of Mg in dairy cows' organisms affects nervous and muscle systems functioning. Before using feed additive for mycotoxins managing, Mg concentration in the EG was below the reference limits. Two months after using feed additive for mycotoxins managing, Mg concentration increased. Jovaišienė *et al.* (2016) together with a group of scientists observed that feed additive for mycotoxins management did not have a notable effect on Mg concentration variation. However, the current result is in contrast with the experiments of Simion (2010) together with scientist group which suggests that mycotoxins induce hypermagnesemia (Simion *et al.*, 2010; Jovaišienė *et al.*, 2016). The GGT is one of the enzymes that reflect liver functionality. Also, GGT has multiple functions including catalytic transfer of γ-glutamyl groups to amino acids and short peptides and contributes to pancreatic transport of amino acids across cell membranes (Ozer *et al.*, 2008). An increase of GGT parameter can be acknowledged as indicative of liver impairment. In this study, in the EG before using feed additive for mycotoxins managing, the GGT concentration was higher than the reference limits, which suggests a liver disorder. Two months after using feed additive for mycotoxins management, the GGT concentration decreased by 50.64±0.01% and no longer exceeded the reference limits. The results of this study agree with Wang's (2019) results obtained with a group of scientists who monitored liver enzymes AST, ALT and ALP in Holstein cows after feeding with feed contaminated with AFB_1 and found that they were not significantly altered. Also, we agree with Wang *et al.* (2020) that mycotoxins can influence liver function, but its effects fluctuate according to toxin dose and duration. However, in a different study of mycotoxins did not affect the plasma concentrations of GGT, AST, ALT or ALP in dairy cows (Jovaišienė *et al.*, 2016; Wang *et al.*, 2019).

IgA is one of the immunoglobulins that has a crucial part in the defensive mechanism of organism and is imperative to immunity. In this study, after two months of using feed additive for mycotoxins managing in CG, IgA concentration decreased, but in the EG IgA it increased. The results of this study agree with the research of other scientists when IgA decreased in blood serum of cows fed with feed contaminated with mycotoxins. According to scientists, a decrease of IgA in blood serum is generated by immunosuppressing toxins produced by *Fusarium* (Korosteleva *et al.*, 2007; Wang *et al.*,

2019; Jovaišienė *et al.,* 2016). The study displayed that the cow group which received feed additive for mycotoxins management had higher of IgA concentration. When stress factor (in this case mycotoxins) is present, adrenocorticotropin hormone excretion from hypophysis is stimulated, then steroid hormone cortisol production from adrenal cortex increases (Korosteleva *et al.*, 2007). In this study, feed additive for mycotoxins managing had a reliable effect on cortisol in the EG, and in the CG the incurred stress (caused by mycotoxins in rations) escalated.

In order to include as much cow health indicators as possible, feed additive for mycotoxins management effect on the reproduction system status was studied. In the CG, there was a 12.5% higher diagnose rate of hypofunctioning ovaries than in the EG, although cyst recognition in cows' ovaries was by 6.1% higher in the EG than in the CG. While investigating in what way mycotoxins risk management had an influence on ovaries functional disruptions, we disclosed that in the EG more (14.98%) dairy cows had an improved response to cyst treatment protocol than in the CG. This suggests that the implementation of feed additive for mycotoxins management improved the efficiency of cyst treatment. Evaluating clinically healthy cows' response to different stimulation protocols it was determined that the EG showed by 18.02% better reaction to applied ovsynch protocol in comparison with the CG. The study displayed that ZEN in feed credibly increases estrogen and prolactin quantity. According to the scientists, ZEN is a mycoestrogen which is comprised of compounds that have an estrogenic impact and provoke cyst occurrences in ovaries (Mona Mahmoud *et al.*, 2013). Our study complies with this proposition since in the CG 9.6% of dairy cows and 15.7% of cows in the EG were diagnosed with cysts. Moreover, Cortinovis *et al.* (2013) declares that these mycotoxins have direct effects on ovarian cells, altering oocyte maturation and *Fusarium* mycotoxins can alter follicular growth and atresia, ovulation, and puberty onset. We also observed disorders of follicular growth and ovulation.

In the present study was established that SCC rate decreased by 55.37±0.01% after two months of feeding additive for mycotoxins management in the EG. It was the essential criteria demonstrating advanced udder wellness. The results of this study agree with the findings of other researchers where mycotoxin risk management in feed helped to reduce SCC (Kiyothong *et al.,* 2012).

CONCLUSIONS

Research has demonstrated that a decreased level of cortisol and increased immunoglobulin A concentration in an experimental group of dairy cows had a positive effect on their health status. However, other analyzed biochemical blood parameters were a weak indicator in assessing the risk management of mycotoxins (AFB_1, ZEN, DON, and OTA) in feed. Research results demonstrated that applying Ovsynch and controlling risk management of mycotoxins in animal feed it is possible to achieve better efficiency by reducing ovarian functional dysfunction of dairy cows. It was also established that by adding feed additives to reduce mycotoxins in feed, the number of somatic cells in milk can be also reduced.

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