FATE OF MILK YIELD AND FERTILITY PARAMETERS IN HOLSTEIN DAIRY COWS SIMULTANEOUSLY EXPOSED TO ZEARALENONE, DEOXYNIVALENOL AND FUMONISIN B_1 MYCOTOXICITY

C. Uyarlar [1] and O. Yilmaz [2]

[1] Department of Animal Nutrition and Nutritional Diseases, [2] Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03200, Afyonkarahisar, Turkiye
Cangir Uyarlar: https://orcid.org/0000-0002-7803-2454
Oktay Yılmaz: https://orcid.org/0000-0002-9722-5155

Corresponding author's Email: cangiruyarlar@hotmail.com

ABSTRACT

This study was planned to investigate the effect of zearalenone (ZEA), deoxynivalenol (DON) and Fumonisin B1 (FB1) on blood metabolites, milk yield, estrous activity, pregnancy rate and uterine health in 486 heads of Holstein dairy cows. For this purpose, the data collected from the animals were divided into the four periods as follows: feeding with non-contaminated diet for 30 days (pre-contamination period), contaminated diet for 60 days (contamination period), contaminated diet supplemented with mycotoxin adsorbent for 30 days (adsorbent period) and the diet after removing the contaminated feedstuffs for 30 days (non-contamination period). Each feedstuff was analysed in contamination period and at the beginning of the non-contamination period to determine the presence or absence of ZEA, DON and FB1 in the diet. The blood metabolic profile was assessed in 21 animals in 1-10 days in milk, selected through cluster random sampling method while milk yield, estrous activity, pregnancy rate and prevalence of metritis/endometritis were compared among all four periods. The milk yield ($P \leq 0.001$) and milk fat ($P \leq 0.05$) in the contaminated period were low as compared to other periods. The concentrations of beta-hydroxybutyric acid ($p \leq 0.001$), non-esterified fatty acids ($P \leq 0.05$), gamma-glutamyl transferase ($P \leq 0.05$), and blood urea nitrogen ($P \leq 0.001$) parameters were significantly higher, while the glucose ($P \leq 0.001$) and triglycerides ($P \leq 0.05$) were lower in the contamination period, as compared to adsorbent and non-contamination periods. The pregnancy rate through artificial insemination decreased significantly during the contamination period ($P \leq 0.05$) as compared to pre-contamination period. The overall pregnancy rate in the pre-contamination period was also significantly decreased as compared to the adsorbent and non-contamination periods ($P \leq 0.001$). The percentage of type III anestrus were significantly lower during the non-contamination period ($P \leq 0.05$). The percentages of cows with metritis during the non-contamination period was significantly lower ($p \leq 0.05$) as compared to the contamination period. It was concluded that the routine feed analysis was necessary to determine the presence of ZEA, DON and FB1. Moreover, mycotoxin binder supplementation and/or removing contaminated feedstuffs from the diet under mycotoxicosis conditions may prevent the severe negative energy balance and improve the milk yield and fertility parameters.

Keywords: DON, ZEA, Fuminosins, Metabolic Parameters, Milk Yield, Fertility, Holstein

https://doi.org/10.36899/JAPS.2024.3.0749 Published first online March 22, 2024

INTRODUCTION

Mycotoxins are synthesized by microfungi and are of crucial importance in food chains, not only for their ability to severely threaten human and animal health, but also for the economic losses to livestock industries (Buszewska-Forajta, 2020; Yang *et al*., 2020). Indeed, farm animals are considered to be at much higher risk of exposure to mycotoxins than humans (Rodrigues and Naehrer, 2012). It has been shown that poultry, pigs (Dänicke and Winkler, 2015), horses and carnivores are highly sensitive to the toxic effects of mycotoxins (Buszewska-Forajta, 2020). So far approximately 400 mycotoxins have been discovered, many of which are yet to be investigated in detail, and some have received more attention for their economic and toxicological attributes than others (Buszewska-Forajta, 2020). Farm animals are mostly exposed to more than one mycotoxin (Klaric *et al*., 2009). The combination of different mycotoxins can show either synergistic (Buszewska-Forajta, 2020) or antagonistic interactions (Yang *et al*., 2020). Fusarium mycotoxins, trichothecenes, deoxynivalenol (DON), fumonisins and zearalenone (ZEA), all have been shown to affect livestock health

(Zain, 2011) and are of special interest in animal nutrition, since they often co-occur in feedstuffs (Minervini and Dell'Aquila, 2008).

It is generally believed that ruminants have lower susceptibility to mycotoxicosis, and they are less sensitive to mycotoxins due to the metabolization, deactivation and total degradation of toxins by microbiota in the rumen. Since the occurrence of side effects of mycotoxins mostly depend on the exposure time and dose of the toxin, as well as the health status of the animal (Buszewska-Forajta, 2020), many dairy farmers believe that ruminal flora is strongly capable of detoxifying mycotoxins, therefore the negative effects of mycotoxins are largely ignored (Rodrigues, 2014). However, feeding and management strategies of high-yielding cows can reverse the above mentioned-phenomena. According to Rodrigues (2014), genetic selection of dairy cows; feeding with complex diets; changes in the cleavage capacity of rumen flora due to feed composition with a high percentage of protein-rich concentrates; maintaining or increasing biological activity of mycotoxin metabolites; decreases in the toxin degrading capacity of rumen due to impairment of the rumen flora; and, predisposition of metabolic diseases and negative energy balance, all enhance the sensitivity of ruminants to exposure of feeds contaminated with mycotoxins. In Turkiye, dairy breeders are typically required to use high concentrate diets for high yielding cows to meet their high energy and protein requirements due to the properties of the forage which is less digestible and has high NDF (Neutral Detergent Fiber) content. High concentrate diets contain an abundance of starch which is known to disrupt rumen fermentation kinetics. Thus, ruminants are more susceptible to fumonisins present in corn grain, and/or corn industry by-products.

It is hypothesized that diets containing high concentrate to meet energy requirements of ruminants can lead to higher levels of mycotoxin in the rumen which can impair the rumen flora, consequently decreasing performance parameters. Moreover, little is known about the effect of naturally contaminated diets with multiple fumonisins on reproduction, even if the cows are fed a non-contaminated diet. Therefore, this study aimed to present, the detection process of feed-borne mycotoxins, the metabolic profile data of cows fed a diet naturally contaminated with DON, ZEA and fumonisins, added to a mycotoxin-adsorbent or non-contaminated diet and to determine milk yield and fertility performance in dairy cows exposed to these mycotoxins.

MATERIALS AND METHODS

Animals and diets: The data was obtained from 486 Holstein dairy cows in milk which were housed in a commercial dairy farm in Turkiye. The cows were housed in free stall barn which had rubber bedding material and were divided into paddocks according to their daily milk yield. Average daily milk production was 30.50 kg with 189 average days in milk (DIM). The cows producing up to 35 kg a day were milked twice daily, whereas animals producing above 35 kg were milked thrice daily. The voluntary waiting period was 53 DIM. Artificial insemination was performed based on synchronization protocols or standing heat observation. Dry matter intake (DMI) was calculated as the amount of served feed minus the amount of residual feed and recorded daily.

The average milk yield at the start of experiment was 30.50 kg/day of the herd (the pre-contamination period). The animals were fed with total mixed ration (TMR) which were given at Table 1. However, it was seen that the milk yield dramatically started to decrease (final drop to 25.70 kg/day), after incorporation of the new batch of feedstuffs purchased (corn grain, corn gluten meal and dried distilled grain soluble) into the diet without any change in the formulation. Hence, samples were collected from the TMR including each feedstuff for mycotoxin (DON, ZEA, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), HT-2 toxin (HT-2), T-2 toxin (T-2), Ochratoxin A (OTA) and Aflatoxin B_1 (Afla B_1)) analysis to determine the reasons beneath the sharp decline in milk yield. When the presence of hazardous amounts of such mycotoxins was diagnosed in feedstuffs, a mycotoxin adsorbent (Mycofix Plus, Biomin GmbH, Austria) was immediately added into the diet. It was determined that the animals were exposed to the mycotoxin contaminated feedstuffs for 60 days (the contamination period). Therefore, it was determined that these contaminated feedstuffs were (corn grain, corn gluten meal and dried distilled grain soluble) purchased and added into the diet 60 days ago. This timeline of mycotoxin contamination was in accordance with the sharp decrease in milk yield of the herd. The animals were then fed a contaminated diet supplemented with mycotoxin adsorbent for an additional 30 days (the adsorbent period). Then, the contaminated feedstuffs (corn grain, corn gluten meal and dried distilled grain soluble) were removed from the diet. The previously contaminated feedstuffs were rebought from a different feed dealer and analysed for the determination of the mycotoxin exposure. When the mycotoxin analysis clarified that the feedstuffs were not contaminated by mycotoxins, the newly purchased feedstuffs were analysed for the nutrient composition. The chemical analysis of the new feedstuffs showed that feedstuffs had similar nutrient content with the removed feedstuffs. Therefore, after this feedstuff substitution (the non-contamination period), the diet formulation was not changed. Therefore, 30 days before the animals were fed the contaminated diet was accepted as the first day of the study (Fig. 1).

All diets were prepared as TMR and administered twice a day (8:00-20:00). All animals were fed the same diet throughout the study, as mycotoxin exposure feedstuffs and their substitutes had similar nutrient compositions. Formulation and chemical composition of the diet is presented in Tables 1 and 2, respectively. The portions of diets were above the animal requirements (125% of the animal requirements according to NRC (2001) in order to measure total dry matter intake. Feed residuals were collected right before the morning feeding and weighed. All feedstuffs were sampled and TMR samples were taken weekly. Chemical compositions of all samples were analysed according to Weende analysis methods (crude protein, crude fat, crude cellulose, crude ash and dry matter) (AOAC, 1990). Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) contents of the feed samples were analysed according to Van Soest *et al*. (1991). Dietary contents of al mycotoxins were analysed by a private laboratory (Multimycotoxin method with HPLC-MS/MS and isotopic labelled internal standards as described by the "AD-SOP 31", Romer Labs Diagnostic GmbH, Tulln, Austria).

Measurement of milk yield and milk composition and metabolic profile: The individual milk yields of the animals, which were taken from an automatic milking system (DairyPlan, GEA, Germany), were recorded daily. Moreover, the weekly measurement of routine milk composition (fat, protein and lactose) was performed by Milkoscan (FT-120, FOSS, Denmark). Blood samples were collected from each of 21 animals in 10 days in milk in all periods according to cluster random sampling method (McDermott *et al*., 1994). Sera were harvested from blood samples following the centrifugation at 5.000 rpm for 10 min. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), glucose, blood urea nitrogen (BUN), total cholesterol (Chol) and triglycerides (Trig) were analysed by the commercially available reagents of Cobas C111 analyzer (Roche Diagnostic, Mannheim, Germany). The serum concentrations of non-esterified fatty acids (NEFA, #FA115, Randox Laboratories Ltd., Crumlin, UK) and beta hydroxybutyric acid (βHBA, #RB1008, Randox Laboratories Ltd., Crumlin, UK) were measured by ELISA (ChemWell, 2910, Awareness Technology, Inc., Florida, USA).

Scanning of reproductive parameters: The herd was examined by transrectal ultrasonography (6 MHz, DP 10, Mindray, China) to detect the ovarian activity of cows between 30 and 80 DIM and the pregnancy status (pregnant or nonpregnant) in each of the contamination, adsorbent and non-contamination periods. The artificial insemination (AI) and the pregnancy rates at each period including the pre-contamination period were recorded and calculated via the data obtained from the herd management system (DairyPlan, GEA, Germany). During each period, the follicles which were classified as emergence (4 mm), deviation (9 mm) and ovulation (variable, from 10 to 20 mm) and the presence or absence of corpora lutea or cystic follicular structures, were recorded. Then, the animals were re-monitored by transrectal ultrasonography 10 days later. The cows were diagnosed as cyclic and type I, II or III anestrus as previously described by Peter *et al*. (2009). The uterine health status (healthy, clinical metritic or clinical endometritic) of cows between 10 and 80 DIM was also evaluated by clinical observation and transrectal ultrasonography as mentioned elsewhere (Sheldon *et al*., 2006).

Statistical Analysis: The distribution of normality of data was evaluated by Shapiro-Wilk normality test. The data regarding dry matter intake, milk yield, milk composition, metabolic profile and biochemistry parameters of the cows at the pre-contamination, contamination, adsorbent and non-contamination periods were evaluated for significance using one-way ANOVA and Tukey's test (SPSS 26.0). The differences between percentages of pregnancy rate, pregnancy rate per AI, inseminated cow rate, infected (metritic or endometritic) or healthy cow rate, anestrus rate (Type I, II and III) and cyclic cow rate were compared among those above-mentioned periods using the chi-square test. Statistical significance was set at $P \leq 0.05$.

RESULTS

Mycotoxin content of the feedstuffs: The concentrations of FB_2, T-2, OTA and $AflaB_1$ of feedstuffs and TMR were all determined at low-risk values, or were not determined. However, corn and corn gluten displayed high concentration of DON and ZEA, whereas these were recorded in moderate concentrations in DDGS. TMR showed detectable contents of mycotoxin such as DON, FB_1, FB_2 and Afla B_1 but the concentration of ZEA was at high-risk level. Moreover, FB_1 was detected at moderate risk level in corn and high risk level in corn gluten, whereas DDGS showed moderate concentrations of HT-2 (Table 3).

Dry matter intake and milk parameters: It was observed that DMI measured during the contamination period decreased significantly ($P \leq 0.001$) as compared to the pre-contamination period. However, it again increased significantly in the adsorbent period and remained unchanged during the non-contamination period (Table 4). The milk yield of the herd fed the contaminated diet (25.70 ± 0.72 kg/day) was low ($P \leq 0.001$) as compared to data of animals obtained at pre-

contamination (30.50 ± 0.48 kg/day), adsorbent (30.47 ± 0.43 kg/day) or non-contamination periods (31.86 ± 0.33 kg/day). Thus, there was a 15.73% decrease in milk yield in the contamination period as compared to that pre-contamination period. However, the milk yield did not show any significant difference after the application of the mycotoxin adsorbent or the removal of the contaminated feedstuff when compared to the pre-contamination period. The milk fat content of the animals exposed to the contaminated diet (3.48 ± 0.01%) was lower (P≤0.05) than that detected in animals in the pre-contamination (3.62 ± 0.03%), adsorbent (3.64 ± 0.05%) or non-contamination (3.64 ± 0.06%) periods. However, there were non-significant differences during pre-contamination, adsorbent or non-contamination periods. Similarly, milk protein and lactose contents did not differ significantly among the four periods.

Metabolic blood profile: The concentrations of blood βHBA (P≤0.001), NEFA (P≤0.001), AST (P≤0.05), GGT (P≤0.001) and BUN (P≤0.001) parameters were significantly higher, while GLU and TRIG were lower, in the animals fed the mycotoxin contaminated diet (contamination period) than those detected in the animals during the adsorbent and non-contamination periods (Table 5). However, after supplementation of mycotoxin adsorbent into the diet, these parameters did not show any significant difference from the data obtained from animals fed the uncontaminated diet. However, the concentrations of ALT and CHOL did not differ between animals fed all three diet regimens.

Reproductive parameters: The values for pregnancy rate per AI, overall pregnancy rate and artificially inseminated cow rate is given in Table 6. It can be seen that the pregnancy rate per AI of the herd decreased significantly from 47.60% during the pre-contamination period to 24.60% during the contamination period (P≤0.05). Then, it non-significantly increased 35.50% during the adsorbent period and reached the pre-contamination level (48.80%) during the non-contamination period (P≤0.05). However, the overall pregnancy rate observed during the pre-contamination period (51.90%) decreased non-significantly to 47.20% in the contamination period but significantly to 32.40% and 20.40% (P≤0.001) during the adsorbent and non-contamination periods, respectively. The proportion of cows inseminated also differed during different periods: the inseminated cow rate was lower in the contamination (8.10%) and adsorbent (13.80%) periods as compared to pre-contamination (14.50%) and non-contamination (16.60%) periods (P≤0.001).

The distribution of cows diagnosed as type I, II anestrus and cyclic did not differ significantly between periods. However, the percentage of cows categorized as type III anestrus during the non-contamination (6.80%) period was significantly lower (P≤0.05) as compared to contamination and adsorbent periods (Table 7). The percentages of cows which were categorized as clinical endometritic and healthy did not differ among contamination, adsorbent and non-contamination periods. Furthermore, the percentage of cows with metritis during the non-contamination period did not differ from the adsorbent period but it was significantly lower (p≤0.05) than that recorded during the contamination period (Table 7).

Table 1: Formulation of the experimental diet.

Feedstuff	Amount (%)
Corn Silage	62.35
Alfalfa hay	12.92
Corn Grain	10.02
DDGS	4.12
Corn Gluten Meal	3.98
Cottonseed (Whole)	3.34
Sunflower Meal	1.78
Limestone	0.75
Sodium Bicarbonate	0.37
Salt	0.32
Premix[1]	0.05

Abbreviations: DDGS = Dried Distillers' Grain Soluble (Corn);
[1] Premix composition in 1 kg; 3000000 IU of Vitamin A, 500000 IU of Vitamin D, 9000 mg of Vitamin E, 12000 mg of Mn, 5000 mg of Fe, 50000 mg of Zn, 9000 mg of Cu, 400 mg of I, 75 mg of Co, 250 mg of S.

Table 2: **Chemical composition of the experimental diet.**

Nutrients	Unit	Level
Crude Protein	(DM%)	16.18

Net Energy Lactation	(Mcal/kg)	1.53
Ether Extract	(DM%)	4.3
Crude Ash	(DM%)	7.9
NDF	(DM%)	33.72
ADF	(DM%)	23.65
Ca	(DM%)	0.55
Phosporus	(DM%)	0.35

Abbreviations: NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber

Table 3: **Feed-borne mycotoxin concentrations (ppb) in feedstuffs.**

			Feedstuffs						Ref (ppb)		
Toxins	CG	C	DDGS	S	CGM	CS	A	TMR	Low	Moderate	High
DON	**4167**	*Nd*	1969	*Nd*	**12073**	152	*Nd*	945	≤1000	1000-2000	>2000
ZEA	**2092**	*Nd*	128	*Nd*	**1804**	49.6	*Nd*	200	≤100	100-200	>200
FB_1	1217	*Nd*	518	*Nd*	**3629**	122	*Nd*	210	≤1000	1000-3000	>3000
FB_2	296	*Nd*	61.2	*Nd*	983	*Nd*	*Nd*	51.2	≤1000	1000-3000	>3000
HT-2	19.2	*Nd*	120	*Nd*	*Nd*	66.8	*Nd*	*Nd*	≤100	100-800	>800
T-2	11.6	*Nd*	70.5	*Nd*	*Nd*	*Nd*	*Nd*	*Nd*	≤100	100-800	>800
OTA	*Nd*	*Nd*	3.1	3.1	*Nd*	*Nd*	*Nd*	*Nd*	≤100	100-300	>300
$AflaB_1$	*Nd*	*Nd*	*Nd*	*Nd*	0.62	*Nd*	*Nd*	0.62	≤5	5-20	>20

Abbreviations: DON = deoxynivalenol; ZEA = zearalenone; FB_1 = fumonisin B_1; FB_2 = fumonisin B_2; HT-2 = HT-2 toxin; T-2 = T-2 toxin; OTA = Ochratoxin A; Afla B_1 = Aflatoxin B_1; CG = corn grain; C = cottonseed (whole, delinted); DDGS = dried distillers' grain soluble; S = sunflower meal; CGM = corn gluten meal; CS = corn silage; A = alfalfa; TMR = total mix rations; Nd = not determined

Table 4: **Dry matter intake (kg/day), milk yield (kg/day) and milk composition (%) (fat, protein and lactose) measured at pre-contamination, contamination, adsorbent and non-contamination periods.**

		Mycotoxin exposure periods			
Parameter	Pre-contamination	Contamination	Adsorbent	Non-contamination	*P*-value
Dry Matter Intake (kg/d)	23.88±0.22[a]	20.59±0.15[b]	23.05±0.24[a]	24.18±0.24[a]	0.000
Milk yield (kg/d)	30.50±0.48[a]	25.70±0.72[b]	30.47±0.43[a]	31.86±0.33[a]	0.000
Milk fat (%)	3.62±0.03[a]	3.48±0.01[b]	3.64±0.05[a]	3.64±0.06[a]	0.011
Milk protein (%)	3.21±0.02	3.19±0.04	3.20±0.03	3.21±0.04	0.995
Milk lactose (%)	4.62±0.02	4.63±0.03	4.65±0.02	4.63±0.04	0.868

[a-b] Values within a row with different superscripts differ significantly at P≤0.05.

Table 5: **The concentrations of blood beta-hydroxy butyric acid (mmol/L), non-esterified fatty acid (mmol/L), aspartate amino transpherase (U/L), alanine transpherase (U/L), gamma glutamile transpherase (U/L), glucose (mg/dL), blood urea nitrogene (mg/dL), cholesterol (CHOL) and triglycerides (TRIG) of the animals fed by contaminated and mycotoxin adsorbent supplemented and non-contaminated diets (mean ± SEM).**

		Mycotoxin exposure periods		
Parameter	Contamination	Adsorbent	Non-contamination	*P*-value
βHBA (mmol/L)	1.190±0.12[a]	0.720±0.04[b]	0.500±0.06[b]	0.000
NEFA (mmol/L)	0.950±0.13[a]	0.500±0.18[b]	0.310±0.16[b]	0.000
AST (U/L)	122.48±6.22[a]	105.32±4.25[b]	98.14±5.05[b]	0.048
ALT (U/L)	38.65±2.28	28.47±1.13	29.33±2.09	0.635
GGT (U/L)	48.87±2.21[a]	30.81±3.23[b]	33.74±3.79[b]	0.000
GLU (mg/dL)	44.25±2.78[a]	60.87±4.65[b]	64.56±3.36[b]	0.000
BUN (mg/dL)	19.25±3.55[a]	11.15±2.58[b]	12.32±0.45[b]	0.000

CHOL (mg/dL)	178.89±19.86	205.82±8.78	200.00±12.12	0.059
TRIG (mg/dL)	6.89 ± 2.35[a]	13.75 ± 1.17[b]	15.49 ± 2.68[b]	0.025

Abbreviations: βHBA = Beta-hydroxy butyric acid; NEFA = Non-esterified fatty acid; AST = Aspartate amino transpherase; ALT = Alanine transpherase; GGT = Gamma glutamile transpherase; GLU = Glucose; BUN = Blood urea nitrogene; CHOL = Cholesterol; TRIG = Triglyceride.
[a-b] Values within a row with different superscripts differ significantly at P≤0.05.

Table 6: **Pregnancy rate per artificial insemination (AI) (%), overall pregnancy rate (%) and AI rate (%) of cows at the end of each pre-contamination, contamination, adsorbent and non-contamination periods.**

		Mycotoxin exposure periods			
Parameter	Pre-contamination	Contamination	Adsorbent	Non-contamination	*P*-value
Pregnancy rate per AI	47.60[a]	24.60[b]	35.50[ab]	48.80[a]	≤0.05
Overall pregnancy rate	51.90[a]	47.20[a]	32.40[b]	20.40[c]	≤0.001
AI rate	14.50[a]	8.10[b]	13.80[ab]	16.60[a]	≤0.001

Abbreviations: AI = Artificial insemination.
[a-c] Values within a row with different superscripts differ significantly at P≤0.05.

Table 7: **The distribution of cows diagnosed as type I, II and III anestrus or cyclic, clinical metritic and endometritic or healthy in each, contamination, adsorbent and non-contamination periods.**

		Mycotoxin exposure periods		
Parameter	Contamination	Adsorbent	Non-contamination	*P*-value
Anestrus Type (%)				
Type I	10.70	12.40	14.90	>0.05
Type II	8.90	11.13	17.60	>0.05
Type III	28.60[a]	23.70[a]	6.80[b]	≤0.05
Cyclic	51.80	52.50	60.80	>0.05
Uterine Health (%)				
Metritis	12.80[a]	4.70[ab]	1.10[b]	≤0.05
Endometritis	15.40	10.90	17.40	>0.05
Healthy	71.80	84.40	81.50	>0.05

[a-b] Values within a row with different superscripts differ significantly at P≤0.05.

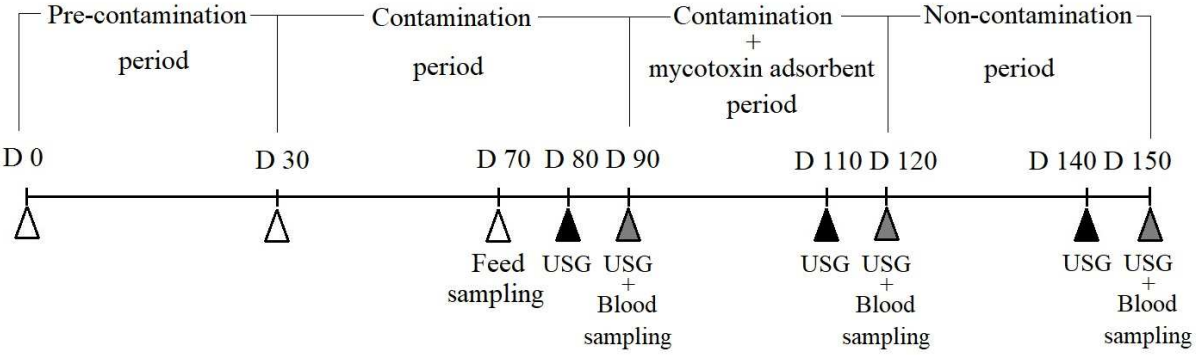

Fig. 1. **The procedure of feed and blood sampling and the transrectal ultrasonography monitoring during contamination, adsorbent and non-contamination periods. Abbreviations: D = Day; USG = Ultrasonography.**

DISCUSSION

The data obtained in this study provide new evidence that multiple mycotoxin exposure may play an important role in milk yield and especially in the fertility performance of dairy cows. It has been documented that corn grain (Abnet, 2007; Silva *et al*., 2020), corn gluten meal (Rodrigues and Naehrer, 2012), corn silage (Aragon *et al*., 2011), DDGS (Rodrigues and Chin, 2012), straw and silage (Duffield, 2000) are the most susceptible feedstuffs in which Fusarium species generally produce mycotoxins such as DON, ZEA and fumonisins (Rodrigues, 2014). The present study demonstrated the effect of Fusarium mycotoxins in feedstuffs with relevance to corn grain or corn by-products. Aflatoxins are also of interest due to their carcinogenic and mutagenic effects in humans (Abnet, 2007). The European Union limits aflatoxin B_1 levels to 20 ppb for feed material (European Commission, 2002). In the present study, the Afla B_1 level was well within accepted EU limits and Afla B_1 at the 0.62 ppb level was detected in only corn gluten and TMR. Perhaps many farmers do not notice that their farm may be susceptible to other mycotoxins, when the Afla B_1 levels are detected within accepted EU limits or are not detectable level. The present study revealed that the farms might be suffering from other mycotoxins even when the concentrations of Afla B_1 in feedstuffs were within accepted limits. Hence, in future the data within accepted limits for ZEA, DON and fumonisins should also be routinely analysed for better mycotoxin management.

Some reports (Charmley *et al*., 1993; Winkler *et al*., 2014; Ismail *et al*., 2020) showed that DMI and milk yield of dairy cattle were not affected by mycotoxin intake. Nevertheless, mouldy odour caused by fungi reduces feed intake and prolongs feeding times due to the sensitivity of cattle to the taste and smell of moulds (Klaric *et al*., 2009; Rodrigues, 2014). Other studies reported significantly reduced feed intake from a mixture of aflatoxins (Korosteleva *et al*., 2007) In the present study, if DMI reduction had occurred due to the diet formulation which was formulated as high concentrate (corn grain and corn by-products) to forage ratio to meet the nutrient needs of animals, it would be expected that the reduction in DMI should have been observed in all examined periods. However, it was found that only the cows fed a contaminated diet showed a decrease in DMI, whereas DMI increased during the adsorbent period and did not change during the non-contamination period. Mycotoxin adsorbents in different properties such as mineral clay carrier, enzyme or probiotics may adsorb and detoxify mycotoxins (May *et al*., 2000). It is suggested that either co-exposure to mycotoxins or feedstuff contamination with higher concentrations of mycotoxins can lead to a reduction in DMI and mycotoxin adsorbents are capable of preventing this DMI reduction.

It was indicated that cows fed with high DON (6500 μg/kg) tended to produce less milk and lower milk fat than those fed with the non-contaminated diet (Charmley *et al*., 1993; Rodrigues, 2014). Some studies also marked a positive correlation between the presence of mycotoxins and low milk production which was a more than 20% drop in milk production (Ismail *et al*., 2020) and decreased feed intake and reduced milk production (Minervini and Dell'Aquila, 2008). In the present study, a 15.73 % drop in milk yield was evident and the milk yield or milk fat, similar to DMI pattern, increased after application of mycotoxin adsorbent or removing the contaminated feedstuff. Since there is a positive correlation between dry matter intake and milk yield (Korver, 1988; Overton and Waldron, 2004), it can easily be suggested that a decrease in DM intake results in a decrease in milk yield. It appears that the mycotoxins present in the diet cause a decrease in milk production due to reducing DMI. According to Dänicke *et al*. (2002), diet with Fusarium toxins reduces the microbial protein synthesis and the flow of total protein at the duodenum (Dänicke *et al*., 2002). The alterations in microbial composition of the rumen flora might be resulted in changing of short-chain fatty acid profile (Seeling *et al*., 2006) and inhibition of the multiplication of cellulolytic microorganisms in the rumen (May *et al*., 2000). The decrement of milk fat content in the contamination period might be the result of a decline in acetic acid-producing bacteria in the rumen. Therefore, it is suggested that mycotoxins may be responsible for altering the milk fat content by changing the rumen flora and that adding a mycotoxin adsorbent to the contaminated diet may be beneficial to prevent the decline of milk fat content.

The concentrations of blood NEFA and βHBA as well as serum glucose level are good indicators to monitor metabolic status of high producing dairy cattle (Duffield, 2000; Hayirli *et al*., 2002). Blood NEFA and βHBA concentrations significantly increased during the contamination period, whereas the glucose concentrations decreased in the present study. Nevertheless, these two indicators remained low during the adsorbent and non-contamination periods, while the glucose concentrations increased. Indeed, when the milk yield increases under insufficient dry matter intake conditions, such as in the fresh period, elevated NEFA and βHBA concentrations in blood is observed due to lipo-mobilisation of adipose tissue following the homeorhetic adaptation processes against the development of negative energy balance (McArt *et al*., 2013). Accordingly, it is suggested that elevated blood concentrations of NEFA and βHBA are consequences of reduced appetite and the reduction of DMI which are on the results of mycotoxin contamination.

The increase of the concentrations of ALT, AST and GGT enzymes in the blood has been associated with lipid accumulation and cell damage in the liver (Kaneko *et al*., 2008; Sakowski *et al*., 2012). Compatible with these implications, AST and GGT levels significantly increased, whereas ALT non-significantly increased in the contamination period. These results were found to be in line with previous studies (Overton and Waldron, 2004; Seeling *et al*., 2006; Sakowski *et al*.,

2012). This study also indicated that mycotoxin adsorbent supplementation had beneficial effects on the recovery of liver function, it was reported previously (Fushimi *et al*., 2015).

In the present study BUN was significantly higher ($P \leq 0.001$) in the animals fed the mycotoxin contaminated diet (contamination period) than those detected in the animals during the adsorbent and non-contamination periods. It is known that when the conversion rate of microbial proteins to the ammonia in the rumen while microbial protein synthesis decreases (Firkins, 1996), excessive ammonia passes the rumen wall, reaches to the liver and converted to urea (Huhtanen *et al*., 2015). It has been stated that mycotoxins reduce the microbial protein synthesis in rumen (Dänicke *et al*., 2002). It is suggested that the reducing microbial protein synthesis may increase the presence of excessive ammonia and consequently increases the BUN concentrations under mycotoxin contamination.

The concentration of triglycerides in the blood mostly decreases during mild or severe fatty liver syndrome or liver deficiency conditions (Van den Top *et al*., 2005). In the present study, triglyceride concentrations were lower in the contamination period than those detected in the adsorbent and non-contamination periods. It is postulated that a decrement of triglycerides comparable with NEFA and βHBA may cause mild liver damage, since recovery of liver function has been shown by supplementation of mycotoxin adsorbent.

It has been reported that the conception rate of dairy heifers treated with 250 mg of ZEA daily over three estrous cycles decline from 87% to 62% (Weaver *et al*., 1986). This study presented a similar finding that a decline in pregnancy rates was observed from 51.90% in pre-contamination period to 47.20% in contamination period. The reduced pregnancy rates are associated with the negative effects of mycotoxins which are possibly attributable to a toxic mechanism (Duffield, 2000; Minervini *et al*., 2001). Guerrero-Netro *et al*. (2020) studied the effect of naturally contaminated feed on superovulatory response in cows and found that 6 ppm DON did not alter the number of viable embryos recovered on day 7 but de-epoxy deoxynivalenol (DOM-1) reduced the motility of spermatozoa in vitro. Moreover, Yousef *et al*. (2017) reported that ZEA disrupted the normal interaction between sperm and bovine oviductal epithelial cells, hence negatively affecting sperm survival. It is suggested that the reduced pregnancy rate might be due to not only the toxigenic effect of mycotoxin on embryo or spermatozoa but also the indirect effect of mycotoxin in terms of reduced feed intake and impaired metabolic status. Mycotoxin adsorbents have beneficial effects in reducing mycotoxin absorption from the intestines of cattle, maintaining endocrine homeostasis and reversing hepatic effects (Fushimi *et al*., 2015).

In the present study, inseminated cows during the adsorbent period displayed a pregnancy rate of 35.50%. Zouagui *et al*. (2017) reported that the cows fed by ZEA, DON, diacetoxyscirpenol and fumonisins contaminated diet supplementation with mycotoxin adsorbent showed 35% pregnancy rate as compared to 19% in non-supplemented cows. In the present study, it was expected that the overall pregnancy rate would be increased during mycotoxin adsorbent period and remained the same or increased during the non-contamination period. However, a dramatic decline in the pregnancy rate was observed throughout the study. In this study, a continuous feeding and management process of the herd was adopted instead of simultaneously treated different diet groups, therefore comparable results would not have been obtained. Moreover, it is believed that the data obtained in this study is valuable to discuss what a herd might experience when the farm faces a co-exposure of fumonisins. Accordingly, it was seen that the artificial insemination rate was the lowest during the contamination period and remained stable during the adsorbent and non-contamination periods. The cows examined for pregnancy diagnosis at the end of the adsorbent period were the animals which were inseminated at the contamination and/or adsorbent period. This was the eligible pattern for the pregnancy diagnosis in the non-contamination period. Therefore, it is suggested that comparatively higher number of cows may be included in synchronization programs in order to obtain reasonable pregnancy rates in mycotoxin contaminated herds fed with mycotoxin adsorbent.

In the present study, a higher percentage of type III anestrus was detected during the contamination period which was inconsistent with the results reported by Silva *et al*. (2000) who indicated that ZEA (300 ppb) affected neither morphometric parameters nor follicle diameter in Nellore heifers. However, Mona *et al*. (2013) reported that a diet containing aflatoxin (19.7 ppb) and ZEA (400 ppb) resulted in animals having follicular cysts. Type III anestrus condition continued during the adsorbent period in this study. ZEA and its metabolites are detectable in bovine follicular fluid (Takagi *et al*., 2008). About 50% of ZEA present in plasma is present in follicular fluid, while an increase of 1 ng/ml DON in plasma is paralleled by an increase of 1.5 ng/ml DON (Winkler *et al*., 2014). Circulating ZEA and its metabolites may affect the ovarian antral follicles by inducing apoptosis of granulosa cells, leading to reduced anti-Müllerian hormone secretion from the granulosa cells of atretic antral follicles (Fushimi *et al*., 2014). Moreover, Nakamura and Kadokawa (2015) reported that ZEA and all its metabolites suppressed LH secretion from the bovine anterior pituitary cells via G-protein-coupled receptor 30 (GPR30) in vitro. The presence of cystic ovaries in the herds contaminated with mycotoxin extends the service period as late as 270 days (Zain, 2011). It is suggested that co-exposure of mycotoxin may play a key role concerning cystic ovarian disease in cows and additional time is needed for decline in the incidence of ovarian follicular cysts. Mycotoxins disrupt not only the ovarian cycles (Rodrigues, 2014) but also the immune system and inflammatory responses in cattle (Marczuk *et al*., 2012; Rodrigues, 2014). In the present study, the contamination period had high percentages of cows which were categorized as having clinical metritis. Baranski *et al*. (2021) also reported that

uterine infection was correlated with blood ZEA concentrations in cows. It is suggested that the immunosuppressive effects of mycotoxins, as well as impaired metabolic status due to reducing DMI, may predispose the cows to uterine infections.

Conclusions: In conclusion, this study indicates that co-exposures of ZEA, DON and fumonisins reduce milk yields by reducing DMI and impairing metabolic status. In dairy cows fed a mycotoxin adsorbent to counteract a contaminated diet, although the adverse effects of decreased DMI, milk yield and fertility parameters attributable to mycotoxin contamination were ameliorated in the short term, it should be taken into consideration that at least 90 days may be needed after the end of the contamination period to regain reasonable fertility parameters.

Ethics approval: All animal-related procedures were approved by the local ethic committee (approval AKUHADYEK 49533702/24).

Disclosure statement: No potential conflict of interest was reported by the authors.

REFERENCES

Abnet, C. C., (2007). Carcinogenic food contaminants. Cancer Invest. 25: 189–196. doi:10.1080/07357900701208733

Aragon, Y. A., I. Rodrigues, U. Hofstetter and E. M. Binder (2011). Mycotoxins in silages: occurrence and prevention. Iranian J. Anim. Sci., 1: 1–10. Available at https://journals.iau.ir/article_513392_1f721a2b37f1c81bc09cfe1b05d6c3e5.pdf (Accessed August 2023)

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. 15th Ed. Virginia (USA). 69-83 pp.

Baranski, W., M. Gajecka, L. Zielonka, M. Mróz, E. Onyszek, K. E. Przybyłowicz, A. Nowicki, A. Babuchowski and M. T. Gajecki (2021). Occurrence of zearalenone and its metabolites in the blood of high-yielding dairy cows at selected collection sites in various disease states. Toxins. 13(446): 1-12. https://doi.org/10.3390/toxins13070446.

Buszewska-Forajta, M. (2020). Mycotoxins, invisible danger of feedstuff with toxic effect on animals. Toxicon. 182(2020): 34–53. doi:10.1016/j.toxicon.2020.04.101

Charmley, E., H. L. Trenholm, B. K. Thompson, D. Vudathala, J. W. Nicholson, D. B. Prelusky and L. L. Charmley (1993). Influence of level of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. J. Dairy Sci. 76: 3580–3587. doi:10.3168/jds.S0022-0302(93)77697-3

Dänicke S., D. Gadeken, K. H. Ueberschar, U. Meyer and H. Scholz (2002). Effects of Fusarium toxin contaminated wheat and of a detoxifying agent on performance of growing bulls, on nutrient digestibility in wethers and on the carryover of zearalenone. Arc. Tierernahr. 56: 245–261. doi:10.1080/00039420214347

Dänicke, S. and J. Winkler (2015). Invited review: Diagnosis of zearalenone (ZEN) exposure of farm animals and transfer of its residues into edible tissues (carry over). Food Chem. Toxicol. 84: 225-249. doi:10.1080/00039420214347

Duffield, T. (2000). Subclinical ketosis in lactating dairy cattle. Vet. Clin. N. Am. Food Anim. Pract. 16(2): 231-253. doi:10.1016/S0749-0720(15)30103-1

European Commission. 2002. Undesirable substances in animal feed. Directive 2002/32/Ec Of The European Parliament And Of The Council, Official Journal L 140 annex I section II Aflatoxin B1.

Firkins, J. L. (1996). Maximizing microbial protein synthesis in the rumen. J. Nutr. 126(suppl_4): 1347S-1354S. doi:10.1080/02652030701823142

Fushimi, Y., M. Takagi, H. Hasunuma, S. Uno, E. Kokushi, U. Watanabe, J. Liu, M. A. Marey, A. Miyamoto, T. Otoi, E. Deguchi and J. Fink-Gremmels (2014). Application of mycotoxin adsorbent to cattle feed contaminated with zearalenone: urinary zearalenone excretion and association with anti-Müllerian hormone. World Mycotoxin J. 7(3): 367-378. doi:10.3920/WMJ2013.1672

Fushimi, Y., M. Takagi, D. Monniaux, S. Uno, E. Kokushi, U. Shinya, C. Kawashima, T. Otoi, E. Deguchi and J. Fink-Gremmels (2015). Effects of dietary contamination by zearalenone and its metabolites on serum anti-Müllerian hormone: impact on the reproductive performance of breeding cows. Reprod. Domest. Anim. 50: 834-839. doi:10.1111/rda.12599

Guerrero-Netro, H. M., M. H. Barreta, E. Costa, A. Goetten, R. Dupras, L. Mills, J. Koch, V. M. Portela, C. A. Price and Y. Chorfi (2020). Effects of the mycotoxin metabolite de-epoxy-deoxynivalenol (DOM-1) on embryo development and sperm motility in cattle. J. Appl. Toxicol. 41(8): 1180-1188. doi:10.1002/jat.4102

Hayirli, A., S. J. Bertics and R. R. Grummer (2002). Effects of slow-release insulin on production, liver triglyceride, and metabolic profiles of Holsteins in early lactation. J. Dairy Sci. 85(9): 2180-2191. doi:10.3168/jds.S0022-0302(02)74297-5

Huhtanen, P., E. H. Cabezas-Garcia, S. J. Krizsan and K. J. Shingfield (2015). Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. J. Dairy Sci. 98(5): 3182-3196. doi:10.3168/jds.2014-8215

Ismail, Z. B., F. Al-Nabulsi, E. Abu-Basha and W. Hananeh (2020). Occurrence of on-farm risk factors and health effects of mycotoxins in dairy farms in Jordan. Trop. Anim. Health Prod. 52: 2371-2377. doi:10.1007/s11250-019-02166-9

Kaneko, J. J., J. W. Harvey and M. L. Bruss (Eds.). (2008). Clinical biochemistry of domestic animals. 6th Ed. Academic press, Elsevier, Oxford, UK. ISBN: 978-0-12-370491-7. pp. 354-361

Klaric, M. S., Z. Cvetnic, S. Pepeljnjak and I. Kosalec (2009). Co-occurrence of aflatoxins, ochratoxin A, fumonisins, and zearalenone in cereals and feed, determined by competitive direct enzyme-linked immunosorbent assay and thin-layer chromatography. Arh. Hig. Rada. Toksikol. 60: 427–434. doi:10.2478/10004-1254-60-2009-1975

Korosteleva, S. N., T. K. Smith and H. J. Boermans (2007). Effects of feedborne Fusarium mycotoxins on the performance, metabolism, and immunity of dairy cows. J. Dairy Sci. 90: 3867–3873. doi:10.3168/jds.2007-0162

Korver, S. (1988). Genetic aspects of feed intake and feed efficiency in dairy cattle: a review. Livest. Prod. Sci. 20(1): 1-13. doi: 10.1016/0301-6226(88)90049-8

Marczuk, J., K. Obremski, K. Lutnicki, M. Gajecka and M. Gajecki (2012). Zearalenone and deoxynivalenol mycotoxicosis in dairy cattle herds. Polish J. Vet.Sci. 15: 365–372. doi:10.2478/v10181-012-0055-x

May, H. D., Q. B. Wu and C. K. Lake (2000). Effects of the Fusarium spp. mycotoxins fusaric acid and deoxynivalenol on the growth of Ruminococcus albus and Methanobrevibacter ruminantium. Canadian J. Microbiol. 46: 692–699. doi:10.1139/cjm-46-8-692

McArt, J. A., D. V. Nydam, G. R. Oetzel, T. R. Overton and P. A. Ospina (2013). Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Vet. J. 198(3): 560-570. doi: 10.1016/j.tvjl.2013.08.011

McDermott, J. J., Y. H. Schukken and M. M. Shoukri (1994). Study design and analytic methods for data collected from clusters of animals. Prev. Vet. Med. 18(3): 175-191. https://doi.org/10.1016/0167-5877(94)90074-4

Minervini, F., M. E. Dell'Aquila, F. Maritato, P. Minoia and A. Visconti (2001). Toxic effects of the mycotoxin zearalenone and its derivatives on in vitro maturation of bovine oocytes and 17b-estradiol levels in mural granulosa cell cultures. Toxicol. in Vitro 15:489-495. doi:10.1016/S0887-2333(01)00068-6

Minervini, F. and M. E. Dell'Aquila (2008). Zearalenone and reproductive function in farm animals. Int. J. Mol. Sci. 9: 2570-2584. doi:10.3390/ijms9122570

Mona, A. M., T. A. Ghattas, Z. A. L. Amal and E. M. Z. Abed (2013). Effect of mycotoxin on reproductive performance in dairy cattle. Assiut Vet. Med. J. 59: 203-213. doi:10.21608/AVMJ.2013.171877

Nakamura, U. and H. Kadokawa (2015). The nonsteroidal mycoestrogen zearalenone and its five metabolites suppress LH secretion from the bovine anterior pituitary cells via the estradiol receptor GPR30 in vitro. Theriogenology. 84:1342-1349. doi: 10.1016/j.theriogenology.2015.07.014

National Research Council (NRC). (2001). Nutrient requirements of dairy cattle. National Academy Press, Washington, DC. ISBN:0-309-06997-1.

Overton, T. R. and M. R. Waldron (2004). Nutritional management of transition dairy cows: strategies to optimize metabolic health. J. Dairy Sci. 87: E105-E119. doi: 10.3168/jds.S0022-0302(04)70066-1

Peter, A. T., P. L. Vos and D. J. Ambrose (2009). Postpartum anestrus in dairy cattle. Theriogenology. 71(9):1333-1342.

Rodrigues, I. (2014). A review on the effects of mycotoxins in dairy ruminants. Anim. Prod. Sci. 54: 1155-1165.

Rodrigues, I. and L. J. Chin (2012). A comprehensive survey on the occurrence of mycotoxins in maize dried distillers' grain and solubles sourced worldwide. World Mycotoxin J. 5: 83–88. doi:10.3920/WMJ2011.1308

Rodrigues, I. and K. Naehrer (2012). A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins (Basel). 4: 663–675. doi:10.3390/toxins4090663

Sakowski, T., B. Kuczyńska, K. Puppel, E. Metera, K. Słoniewski and J. Barszczewski (2012). Relationships between physiological indicators in blood and yield as well as chemical composition of milk obtained from organic dairy cows. J. Sci. Food Agri. 92: 2905–2912. doi: 10.1002/jsfa.5900

Seeling, K., P. Lebzien, S. Dänicke, J. Spilke, K. H. Sudekum and G. Flachowsky (2006). Effects of level of feed intake and Fusarium toxin-contaminated wheat on rumen fermentation as well as on blood and milk parameters in cows. J. Anim. Physiol. Anim. Nutr. 90: 103–115. doi:10.1111/j.1439-0396.2005.00570.x

Sheldon, I. M, G. S. Lewis, S. LeBlanc and R. O. Gilbert (2006). Defining postpartum uterine disease in cattle. Theriogenology. 65(8): 1516-1530. doi:10.1016/j.theriogenology.2005.08.021

Silva, L. A., M. R. B. Mello, D. O. Pião, L. N. Silenciato, T. C. O. Quadros, A. H. Souza and R. P. Barbero (2020). Effects of experimental exposure to zearalenone on reproductive system morphometry, plasma oestrogen levels, and oocyte quality of beef heifer. Reprod. Domest. Anim. 56: 775-782. doi:10.1111/rda.13917

Takagi, M., S. Mukai, T. Kuriyagawa, K. Takagaki, S. Uno, E. Kokushi, T. Otoi , A. Budiyanto, K. Shirasuna, A. Miyamoto, O. Kawamura, K. Okamoto and E. Deguchi (2008). Detection of zearalenone and its metabolites in naturally contaminated follicular fluids by using LC/MS/MS and in vitro effects of zearalenone on oocyte maturation in cattle. Reprod. Toxicol. 26: 164-169. doi:10.1016/j.reprotox.2008.08.006

Van den Top, A.M., A. Van Tol, H. Jansen, M. J. Geelen and A. C. Beynen (2005). Fatty liver in dairy cows postpartum is associated with decreased concentration of plasma triacylglycerols and decreased activity of lipoprotein lipase in adipocytes. J. Dairy Res. 72(2): 129-137. doi:10.1017/S0022029905000774

Van Soest, P. J., J. B. Robertson and B. A. Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10): 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2

Weaver, G. A., H. J. Kurtz, J. C. Behrens, T. S. Robison, B. E. Seguin, F. Y. Bates and C. J. Mirocha (1986). Effect of zearalenone on the fertility of virgin dairy heifers. Am. J. Vet. Res. 47:1395-1397. Available at https://pubmed.ncbi.nlm.nih.gov/2942065/ (Accessed May 2023)

Winkler, J., S. Kersten, U. Meyer, U. Engelhardt and S. Dänicke (2014). Residues of zearalenone (ZEN), deoxynivalenol (DON) and their metabolites in plasma of dairy cows fed Fusarium contaminated maize and their relationships to performance parameters Food Chem. Toxicol. 65: 196-204. doi:10.1016/j.fct.2013.12.020

Yang, C., G. Songa and W. Lim (2020). Effects of mycotoxin-contaminated feed on farm animals. J. Hazard. Mater. 389: 122087. doi:10.1016/j.jhazmat.2020.122087

Yousef, M. S., M. Takagi, A. K. Talukder, M. A. Marey, R. Kowsar, A. K. Abdel-Razek, T. Shimizu, J. Fink-Gremmels and A. Miyamoto (2017). Zearalenone (ZEN) disrupts the anti-inflammatory response of bovine oviductal epithelial cells to sperm in vitro. Reprod. Toxicol. 74: 158-163. doi:10.1016/j.reprotox.2017.09.012

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc. 15(2): 129-144. doi:10.1016/j.jscs.2010.06.006

Zouagui, Z., M., Asrar, H. Lakhdissi and E. H. Abdennebi (2017). Prevention of mycotoxin effects in dairy cows by adding an anti-mycotoxin product in feed. J. Mater. Environ. Sci. 8(10): 3766-3770. Available at https://www.jmaterenvironsci.com/Document/vol8/vol8_N10/396-JMES-1529-Zouagui.pdf (Accessed at September 2023).