EFFECT OF USING MYCOTOXINS DEACTIVATOR WITH NATURALLY MYCOTOXINS CONTAMINATED OR NOT CONTAMINATED CORN SILAGE ON LACTATING COWS PERFORMANCE

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SUMMARY

Mycotoxins especially aflatoxin B_1 and zearalenonecausea wide range of harmful effects in farm animals. The problems of mycotoxins are not only just end in the harmful effect on animal performance but also many of them become concentrated in milk and can pose a threat to human health. So, this study aimed to investigate the effect of using mycotoxin deactivator inlactating animal ration to reducing the harmful effect of micotoxins on animal performance and milk safety. A total of twentyfour primiparous and multiparous Holstein lactating cows were assigned to two groups of twelve cows each. The experiment was extended for one month and the animals were fed total mixed ration (TMR) in quantities suffusion to provide about 10% in excess of the expected daily intake for *ad libitum* consumption. The control group was fed TMR containing about 10% high microbiologically quality corn silage (normal silage) + mycotoxindeactivator (MD) product, while the second group was fed TMR containing about 10% low microbiologically quality corn silage (naturally contaminated with mycotoxins) + MD product. A significantly ($P<0.05$) reduction in ruminal viable total bacteria and cellulytic bacteria count were observed in the group fed mycotoxin contaminated silage compared to the control group. Acetate and propionate concentration were significantly ($P <0.05$) lower in ruminal fluid of the cows fed mycotoxin contaminated silage than those in control group. Feeding mycotoxin contaminated silage significantly ($P<0.05$) decreased DM, CF, NFC and ADF digestibility compared to control group. While insignificant ($P>0.05$) differences were observed in digestibility of OM, CP, EE and NDF. No significant effect on the concentration of total protein, globulin, A/G ratio, bilirubin concentration and ALT and AST activity. Cows fed contaminated corn silage showed a decrease in the averagefeed intake andmilk production. Feeding contaminated corn silage resulted inincreased aflatoxin M_1 in milk. These results support that the hygienic quality of silage is more important than using mycotoxins deactivator for animal performance, even with using low percent of contaminated feed and maintain the level of total aflatoxin and aflatoxin B_1 concentration lower than the permissible limits.

Keywords: *nutrientsdigestibility, performance, rumen ecology, VFA concentration, milk safety.*

INTRODUCTION

Consumption of mycotoxin contaminated feeds induce: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (due to immune-suppression), and reduced reproductive capacities (Fink-Gremmels and Malekinejad, 2007 and Whitlow and Hagler(2005) which leads to economic losses (Wu, 2006).Aflatoxine B_1 (AFB_1) reduced cellulose breakdown and production of VFAs and NH_3 both in *in vivo* and *in vitro* rumen model systems (Mertens and Wyatt, 1977).

The problems of mycotoxinsare not only just end in reducedanimal feed andreduceanimal performancebut also manyof thembecome concentrated in meat, egg and milk as an animal productand can pose a threat to human health, which explains the major concern of food and feed industries in preventing them from entering the food chain (Akande*et al.*, 2006).

The hygienic quality of silage is important for animal health, animal production and food quality and safety. Silages with poor aerobic stability can be found on many farms because of slow filling rates or inadequate packing densities at the time of ensiling. In addition, removal of inadequate amounts of silage during feedout and poor management at the face of the silo exposes the silage mass to prolonged contact

with air. Consequently, lactate-assimilating yeasts (e.g., *Candida*, *Endomycopsis*, *Hansenula*, and *Pichia*; Woolford, 1990) degrade lactic acid to carbon dioxide and water and produce excessive heat that leads to a loss of nutrients. Degradation of lactic acid also increases the pH of the silage to a level that allows opportunistic bacteria (e.g., *Bacilli*; McDonald *et al.,* 1991) and molds (e.g., *Aspergillus*, *Fusarium*, and *Penicillium*; McDonald *et al.,* 1991) to grow and further reduce silage quality and possibly also the formation of toxic metabolic products by moulds (mycotoxins) significantly affect the quality of the silage, possibly even to the extent that it becomes unfit for use as feed.

It is very important to control the total ration mycotoxins through modern detoxification methods, based on the utilization of dietary supplements such as absorptive materials that can bind the most types of mycotoxins(yeast cell walls, clays and charcoal, etc.). Yeast cell walls constituents such as mannan oligosaccharides (MOS) and beta-glucan have shown the ability to bind aflatoxins (Zaghini*et al.,* 2005) and zearalenone(ZEA;Yiannikouris*et al.,* 2004), respectively. Clays have shown the ability to bind ZEA (Sabater-Vilar*et al.,* 2007) but have no affinity for trichothecenes (Sabater-Vilar*et al.,* 2007). Absorptive agents have been the object of many studies. Hydrated sodium aluminosilicates (HSCAS) have shown a high affinity for aflatoxin B_1 and the ability to reduce its growth inhibitory effects in chicks (Phillips *et al.,* 1988). In a study with piglets fed diets contaminated with aflatoxins, different clays (including zeolite, sepiolite, and bentonite) improved animal performance (Schell *et al.,* 1993).

So**,** the objective of the current study was to investigate effect of adding mycotoxindeactivatortolactating cows rations with mycotoxins contaminated or not contaminated corn silage on feed intake, rumen ecology, digestibility, blood metabolites and productive performance.

MATERIALS AND METHODS

This study was conducted at private farm,Benisuef, Egypt, Animal Reproduction Research Institute, Agricultural Research Center and Research Laboratories of Animal Nutrition, Department of Animal Production, Faculty of Agriculture, AinShams University.

Mycotoxins deactivator:

The mycotoxins deactivator(MD) contained anti-fungal agents and liver activator such as sorbic acid 0.05%, citric acid 0.75%, calcium propionate 10.5%, copper sulphate5%andinactivated yeast (*Saccharomyces Cerevisiae*) 2% as a source of yeast cell walls and sepiolite 41.7%, bentonite 40% as toxin adsorbent agent. The MD was added to the ration of the two groups as feed additive.

Animalsmanagement and experimental design:

Twenty-four primiparous and multiparous Holstein lactating cows(620 Kg average body weight, 32kg average daily milk yield and 126 average days in milk(DIM)) were assigned to two groups of twelve cows each, according toproductivity, DIM andlactation season, the animals were each randomly allocated to one of the two dietary treatments. The experiment was extended for one month and the animals were fed total mixed ration (TMR, Table 1) in quantities to provide about 10% in excess of the expected daily intake for *ad libitum* consumption. The control group was fed TMR containing about 10% high microbiologically quality corn silage + MDproduct, while the second group was fed TMR containing about 10% low microbiologically quality corn silage (naturally contaminated with mycotoxins)+ MDproduct. Each group was placed in a shaded pen equipped with free stalls. The diets wereformulated to cover NRC requirement for dairy cattle (NRC, 2001).Cows were fed as a group with free access to water.Feed intake and feed refusals were recorded daily for each group and DM intake was calculated.

Sampling and analytical methods:

Feed and fecal samples:

Samples of corn silages and TMRs were collected weekly and composed to get one sample from each feedstuff. Feed samples and fecal matter samples were dried at 60°C for 48 h then ground to pass a 1mm screen in a Wiley mill before analyzed. Samples of TMR were analyzed for dry matter (DM), organic matter (OM), crude fiber (CF), ether extract (EE) and crude protein (CP) according to AOAC, (2000). The NDF and ADF were determined according to Van Soest*et al.* (1991). Non-fiber carbohydrate (NFC) was calculated according to the following equation:

NFC % = 100− (%NDF+%CP + %fat + %ash) according to NRC (2001).

Table (1). Formulation and chemical composition of the experimental total mixed ration.

Ingredient Total mixed ration formulation, % as fed	Control TMR***	Contaminated TMR
Egyptian clover (Berseem	71.43	71.43
Non contaminated corn silage	10.71	0
Contaminated corn silage	0	10.71
Yellow corn grain, ground , dry	7.77	7.77
Cotton seed cake	2.68	2.68
Soybean meal 44%	5.09	5.09
Wheat bran	1.81	1.81
Lime stone	0.11	0.11
Salt	0.11	0.11
Premix *	0.05	0.05
Detoxification agent	0.04	0.04
Sodium bicarbonate	0.2	0.2
Chemical composition (g/kg DM)		
Organic matter (OM)	880.10	875.06
Crude protein	170.53	170.21
Ether extract (EE)	69.37	70.87
Non fiber carbohydrate (NFC)	310.43	292.50
Neutral detergent fiber (NDF)	329.78	341.47
Acid detergent fiber (ADF)	199.93	210.05
NE_L (Mcal/kgDM)**	1.57	1.57

** Contained 10000000 IU Vitamin D3, 2500000 IU Vitamin A, 35000 mg Vitamin E, 1000 mg Biotin, Zinc 100000 mg, Mn 80000 mg, Cu 30000 mg, I 800 mg, Co 400 mg , Se 300 mg , $CaCO_3$ to 3 kg).*
***Calculated using published values of feed ingredients (NRC, 2001).*
**** TMR: Total mixed ration.*

Table (2).Mycotoxins contents of normal and contaminated corn silage as well as the experimental total mixed rations

Ingredient	Control TMR	Contaminated TMR	normal silage	contaminated silage
Mycotoxins concentrations µg/kg DM:				
Total mycotoxins	2.76	5.05	14.83	39.71
Aflatoxin B_1 (AFB_1)	1.85	1.98	4.9	6.3
Zearalenone	131.79	142.27	195	309
Ochratoxin	5.84	6.40	11.2	17.21

Feed samples were subjected to analyze the presence of the most important mycotoxins affecting animal performance and health, namely, total aflatoxin, aflatoxinB_1,zearalenone, ochratoxin using ELIZA according to Berthiller*et al.*(2007).

Milk sampling and analysis:

Cows were milked three times daily at 4 am, 12 pm. and 8 pm using De-laval milk manager model Sortie. Milk yield for all cows were determined daily, milk samples were obtained once every weeks from each cow for the three consecutive milking and pooled within cow relative to production to obtain one composite milk sample per animal and stored at +4 °C until subjected to chemical analyses. Milk samples were analyzed for total solids, fat, true protein, solid-not-fat, lactose and milk-urea N (MUN) by infrared spectrophotometry (Milko-Scan, FT 6000). While somatic cell counts (SCC) was analyzed using the Fossomatic 5000. Aflatoxin M_1 (AFM_1) was determined using ELIZA according tomethods of Berthiller*et al.* (2007). Fat corrected milk (4% fat) was calculated according to equation of Gaines (1928).

$$4\% \text{ FCM} = 0.4 \text{ milk yield (g)} + 15 \text{ fat yield (g)}$$

Blood sampling and analysis:

At the end of experimental period, blood samples were taken from the coccygeal vasculature using the vacutainer system from 10 experimental animals of each group. The blood sample was collected in a clean centrifuge tube,the blood serum was obtained by centrifuging the blood samples 2h after collection at 3500 (rpm) for 20 minutes. Blood serum was transferred into a clean dried glass vials and then stored in deep freezer at -20º C for subsequent specific chemical analysis. Blood serum samples were analyzed using commercial kits. Total serum protein concentrations was determined as described by Weichselbaun (1946), albumin concentrations was determined using methods of Dumas and Biggs (1972)and activity of serum alanintransaminas (ALT) and aspartate transaminas (AST) were determined using ALT and AST kits, respectively based on reaction of Reitman & Frankel (1957).Globulin was calculated by subtraction of total serum protein and serum albumin, while AG ratio was calculated by dividing the value of albumin on the value of globulin in serum.

Rumen liquor sampling and analysis:

At the end of the experiment, rumen liquor samples (about 200 ml) were taken 4 h after morning feeding, from 10 and 8 cows for control and contaminated group using stomach tube connected with a vacuum pump. Samples of rumen liquor were strained through three layers of cheesecloth. Ten ml of strained rumen liquor samples were immediately transferred to the laboratory and the most probable number calculations were used to estimate the numbers of total viable bacterial count and cellulolytic bacterial count in rumen fluid on nutrient broth and cellulose broth medium, respectively (Nikki *etal.,* 2011). The remaining strained samples were acidified with 5 ml of 2 N sulphuric acid (H_2SO_4)to stop microbial activity. The samples were then centrifugated at 3000 rpm for 10 min and the supernatant (100 mL) was taken. The supernatant was kept in aplastic bottle where 5 mL of 1 M H_2SO_4 was added and frozen at -20 ^oC for VFAs analyses. Ruminal liquor samples were analyzed for VFAs by using spectrophotometer, according tothe method of Siedlka*et al.* (2008).

Digestibility trial:

Acid insoluble ash (AIA) was used as an internal marker for determining the digestibility **(**Van Keulen and Young 1977**).** Fourh after the morning feeding, fecal samples were collected for three consecutive days from rectum of each cow. The feces of each cow were mixed thoroughly, and a subsample (500 g) was dried at 60^oC in a forced air oven for 48 h. Dry samples were ground witha Wiley mill (2mm screen). Dry matter excreted in feces was calculated by dividing AIA input in the feeds (grams of AIA/ day) by AIA output in the feces (grams of AIA/ day). The digestibility coefficient of certain nutrients was calculated according to the formula of Van Keulen and Young (1977**):**

$$Digestibility = 100 - \left[100\, x \, \frac{\%\, indicator in\ feed}{\%\, indicator in\ feces} \, x \, \frac{\%\, nutrient in\ feces}{\%\, nutrient in\ feed} \right]$$

Statistical analyses:

The obtained data were statistically analyzedusing ANOVA procedure for a complete randomized designaccording to statistical analysis system (COSTAT) according to Snedecor and Cochran (1982).Repeated-measures data over time and within animal wereaveraged before analysis. Separation between means was carried out by using Duncan Multiple Range Test (Duncan, 1955)**.**Differences are deemed significant when $P \leq 0.05$. The full model tested was:

$$Y_{ij} = \mu + T_i + e_{ij},$$

Where y_{ij} = represents observation, μ=: the overall mean, T_i = effect of treatment (experimental group), e_{ij}: experimental error.

RESULTS AND DISCUSSION

Chemical compositionof the experimental rations and mycotoxinsconcentrations:

The chemical composition of the feed ingredients used in this study is presented in Table (1). The data indicated that both groups were fed on the same formula with replacing the normal corn silage with the contaminated corn silage in contaminated TMR and the slightly differences in the chemical composition may be due to the differences in corn silages chemical composition.The data indicated that total mixed ration nutrientscontents for both groupswere met cows with nutrient requirements according to

NRC(2001).The data of Table (2) indicated that contaminatedcorn silage which fed to the mycotoxins infected groupcontains a high level of total aflatoxin andaflatoxin B_1, which exceed the permissible limits 20 and 5µg/kg DM, respectively, as well as contain 58.5 and 53.8 % more zearalenoneand ochratoxin compared to the non-contaminated corn silage which fed to the control group. While the values oftotal aflatoxin andaflatoxin B_1 contents in TMR for both experimental treatmentswere not exceeded the permissible limits. The Food and Drug Administration(FDA) has established no guidelines for zearalenone andochratoxin in feed, so any contamination issue is dealt with on a case-by-case basis (Henry, 2006).

Populations of ruminal microorganisms and VFA concentrations:

Rumen activity, total bacteria count and cellulytic bacteria count are presented in Table (3). Although, the mycotoxin deactivator used in this study contains a mixture of antifungal agents and toxin adsorbent agents as well as the total aflatoxin and aflatoxin B_1 contents in TMR for both experimental rationswere not exceeded the permissible limits, a significantly ($P<0.05$) reduction in ruminal viable total bacteria and cellulytic bacteria count were observed in the groupfed mycotoxincontaminated silage compared to the control group, which estimated by about 10.77 and 28.57%, respectively. It could be observed that cellulytic bacteria are the most affected populations in rumen bacteria. These results could be due to the toxic effect of mycotoxins on rumen bacteria.May *et al*. (2000)observed that presence of vomitoxin, deoxynivalenolorfusaricacid separately or together caused a depression in *Methanobrevibacterruminantium*microbialactivity.

Total volatile fatty acid concentrations are shown in Table (3). The data revealed that ruminal acetate and propionate concentration were significantly ($P <0.05$) lower in ruminal fluid of the cows fedmycotoxincontaminated silage than those in control group.Also, butyrate concentration tend to be significantly ($P=0.056$) higher in control group compared totreated group. It could be attributed to the negative effect of mycotoxin on total rumen bacteria and cellulyticbacteria counts in this study (Table 2) which depress rumen fermentation activity consequently, decrease VFA's concentration and or the lower capability of MD to decrease the effect of mycotoxins on fermentation. In this connection Tabia*et al.* (2005)foundin *in vitro* study thatacetate concentration was depressed ($P<0.05$) withpatulin(mycotoxin)addition due to a reduction in fiber digestion. Conversely, there was a shift in fermentation with buterate and valerate concentration, which increased significantly with adding patulin. Also Cook *et al.*, (1986)andDiekman and Green, (1992)reported that presents aflatoxin in feed have been induce compromise ruminal function by reducing cellulose digestion, volatile fatty acids (i.e. acetate, propionate and butyrate) production and rumen motility.

Table (3). Effect of experimental treatments on rumen populations andvolatile fatty acids concentrations.

Item	Control TMR	Contaminated TMR	P-value
Number of animal (N)	10	8	
Total bacterial count (cfu x 10^8/ ml)	28.5 ± 0.27^a	25.43 ± 0.3^b	0.053
Cellulolytic bacterial count (cfu x 10^6/ ml)	4.9 ± 0.05^a	3.5 ± 0.08^b	0.0016
Volatile fatty acids concentration			
Acetic, mm/ml	26 ± 0.32^a	21.73 ± 0.5^b	0.0473
Propionic, mm/ml	11.146 ± 0.27^a	7.56 ± 0.16^b	0.0081
Acetate/propionate ratio	2.33	2.87	
Butyric, mm/ml	5.093 ± 0.17^a	3.616 ± 0.09^b	0.0565

Values in a row followed by a different superscripts are significantly different (P<0.05).

The observed adverse effect on rumen population and fermentation parameters in this study may be pointed to that the mycotoxin deactivator had no the capability to totally stop the harmful effect of mycotoxinsin contaminated corn silage. This result could be attributed to that the antifungal agent had no effect on mycotoxins in the rumen and or the lower present of MD in the total ration (0.04%). In this connection, several experiments reported that inorganic adsorbing agents were able to decrease the harmful effect of mycotoxins in ruminant ration when used at high percent, which Kutz*et al.* (2009) usedHSCAS at 0.56%;Galvano*et al.*,

(2001) used HSCAS at 2%; Smith *et al.* (1994)usedbentonite by 2 and 4%and Diaz *et al.*, (2004) used sodium-bentonite at 1.2%.

Nutrients digestibility:

The data of Table (4) showed the effect of experimental diets on nutrients digestibility coefficients.Despite mycotoxins deactivator compounds was added for both experimental treatments and the total aflatoxin and aflatoxin B_1 contents of both experimental rationswere not exceeded the permissible limits.Feeding mycotoxin contaminated silage showed significant ($P<0.05$) decrease forDM, CF, NFC and ADF digestibility compared to feeding non-contaminated silage (control group),while insignificant ($P>0.05$) differences were observed in digestibility of OM, CP, EE and NDF as well as the feeding values as digestible crude protein ant total digestible nutrientsbetween the two experimental groups.

Table (4). Effect of experimental treatments on nutrients digestibility, %.

Item	Control TMR	Contaminated TMR	p-value
Number of animal (N)	10	8	
Dry matter, %	57.54[a]	54.99[b]	<0.001
Organic matter, %	58.589±0.02	58.09±0.03	0.66
Crude fiber, %	63.11±0.04[a]	54.21±0.08[b]	0.003
Crude protein , %	66.053±0.08	66.11±0.02	0.95
Ether extract, %	84.71±0.04	84.89±0.03	0.91
Non fiber carbohydrate, %	48.40±0.06[b]	53.62±0.04[a]	0.045
Neutral detergent fiber, %	49.17±0.02	47.57±0.05	0.31
Acid detergent fiber, %	47.10±0.05[a]	40.75±0.07[b]	0.025
Nutritive Value:			
Digestible crude protein (DCP)	11.24±0.10	11.27±0.11	0.994
Total digestible nutrients (TDN)	58.98±0.65	58.24±0.72	0.774

Values in a row followed by a different superscripts are significantly different (P<0.05).

The great reduction in crude fiber and ADF degradation, 14.10 and 13.48 % respectively in the mycotoxin affected group compared to the control group could be attributed to the great reduction in total rumen bacteria especially cellulytic bacteria count in the present study (Table 3). The results of Tapia *et al*. (2005) support our results which found that digestion of organic matter, acid detergent fiber, non-fiber carbohydrate and crude protein was reduced (*P*<0 .05)withpatulinaddition. Also, Westlake *et al.* (1989) and Mojtahedi (2013) observed that *in vitro* DM degradability (IVDMD) and total gas production were decreased significantly with inclusion of AFB_1 in culture medium. While Auerbach*et al.* (1998) reported a rumen AFB_1 content of 9.5 µg/ml did not modify *in vitro* digestion of alfalfa and VFAs productions. The reasons for this might be the low mycotoxin concentrations in the inoculums media, or high-quality environmental conditions at the research facility, which could result in unpredictable effects.

Blood metabolites:

The effect of feeding ration contaminated with mycotoxins on blood metabolites are presented in Table (5). The data indicated that adding mycotoxins deactivator to the ration of animals fed contaminated corn silage success to some extent to dilute the harmful effect of mycotoxins on some blood metabolites which, there is no significant effect on the concentration of total protein, globulinandA/G ratioas well as non significant increase in ALT and AST activity for group fed contaminated corn silage compared to control group. Although, the observed insignificant results in blood metabolites in this study, the data may be pointed to that the animal fed rations not contaminated recorded for optimum ALT and AST activity compared to the animals fed ration contained contaminated corn silage. These results are in agreement with Bingol*et al.* (2007)who reported that no correlation was found between feeding contaminated feed with aflatoxinand AST activity when studied the influence of aflatoxin levels in forages and concentrate feedstuffs on some serum parameters in goats. On the contrary, negative correlations were observed between feed total aflatoxin content and total proteins, albumin, globulin concentrations, and ALT activities (Bingol*et al.,* 2007).The results of Bingol*et al.* (2007) suggest that there was no liver damage to the goats when consuming 82 to 820µgAFB_1/kg of the diet.While,Applebaum*et al.* (1982) reported higher blood levels of bilirubin andASTin dairy cows consuming contaminated feeds. In addition, dairy cows exhibited decreased milk production, liver

damage and lethargy (Neathery*et al.*, 1980),this inconsistency between results were may be due to differences in animal species, animal ration, physiological status, milk production and types of mycotoxins.

On the other hand,the data indicated that albumin concentration was significantly lower($P<0.05$) in cows fed contaminated corn silage compared to the control group, meanwhile that the control group was in best metabolism status compared to the group fed contaminated silage.This result could be due to that the control group recorded higher DM digestibility (Table 4), which led to more available nutrients for absorption.

Table(5).Effect of experimental treatments on some blood metabolites.

Item	Control TMR	Contaminated TMR	P-value
Number of animal (N)	10	8	
Total protein, g dl^{-1}	6.16±0.09	6.18±0.09	0.96
Albumin, g dl^{-1}	$3.59±0.07^{a}$	$2.95±0.07^{b}$	0.05
Globulin, g dl^{-1}	2.56±0.11	3.23±0.08	0.18
A/G ratio	2.11±0.15	0.99±0.03	0.06
AST, unit l^{-1}	110±6	80±6	0.28
ALT, unit l^{-1}	37±2	85±8	0.08

Values in a row followed by a different superscripts are significantly different (P<0.05).

Feed intake and milk production and composition:

Data of Table (6)showed that although the total aflatoxin and aflatoxin B_1 were not exceeded the permissible limits for both rations, feeding contaminated corn silage with using MD significantly decreased ($P < 0.05$) dry matter intake. This may be attributed to effect of mycotoxin on digestibility which the cows fed contaminated corn silage recorded the lowest DM and CF digestibility (Table 4) compared to control group.The present results are in agreement with findings of Whitlow and Hagler (2008), who observed that feeding dairy cows with contaminated feeds resulted in reducing feed consumption. On the contrary Kutz*et al.* (2009)found that no effects on DMI or milk yield whencontaminated TMR with $AFB_1$was fed to dairy cows.

Also, the data indicated that feeding contaminated corn silage even with using MDsignificantly ($P<0.05$) reduced milk production compared to the control group.The higher milk production of cows in the control group in the present study may be supported with that the control group recorded for higher rumen activity (Table 3), higher nutrients digestibility (Table 4), higher albumin concentration (Table 5) and higherfeed intake (Table 6) which was assumed to supply more nutrients than the group fed corn silage contaminated with mycotoxins.

The present results are in agreement withWhitlow and Hagler (2008)who reported that mycotoxins affect dairy cows by reducing milk production. On the contraryKorosteleva*et al.* (2007) observed that feeding a TMR naturally contaminated with mycotoxins to lactating cows did not reduce milk production. This inconsistency may be due to types and levels of mycotoxinscontamination, ration formula and cow physiological status.

Concerning to milk chemical composition the data of Table (6)indicated that milk lactose was significantly decreased in group fed contaminated corn silage compared to control group. While,there was no significant differences between the two groups in milk protein, fat,total solids and milk urea contentsas well as somatic cell count. Smith *et al.* (1994) found insignificant effect in milk protein and fat percentages due to feeding diet contaminated with $AFB_1$to goats. Also Kutz*et al.* (2009) reported that milk protein and fat percentage were unaffected when dairy cows were fed an aflatoxin contaminated diet.

Concerning effect of experimental treatment on feed conversion, the data of Table (6) indicated that the animal of the control group recorded the best feed conversion as kg DM per kg milk and kg DM per kg FCM 4% compared to the group fed contaminated corn silage. These results could be attributed to that the control group recorded the higher DM digestibility and higher feed intake as well as the higher milk and FCM yield compared to the group fed contaminated corn silage.

Milk safety:

To avoid the risk of aflatoxin ingestion and intoxication, agencies around the world have established acceptable limits for aflatoxin concentration in milk and feeds. In the United States, the FDA stipulated action levels for aflatoxin in raw milk and lactating cow feeds are 0.5 µg/L and 20 µg/kg, respectively (FDA, 2000). The maximum allowable concentration set by the European Commission is 0.05 µg/L of milk (EFSA, 2004).

The results of Aflatoxin M1 concentration,µg kg^{-1} during the experimental period are presented in Table (6). The dataindicated that aflatoxin M_1 concentration in milk of the group fed contaminated corn silage was higher than those in milk of control group during all of the experimental periods (4 weeks). Moreover, the present results indicated thataflatoxin M_1 transfer rate to milk ranged between 1.62 to 2.97% for the control group, while ranged from 14.14 to 18.18% for the group fed contaminated corn silage. These mean that micotoxins deactivator could not decrease transfer rate under feeding contaminated feed. In this connection, Veldman*et al.* (1992) found that AFB_1in the feed materialswastransferred to milk as AFM_1 ranged from 0.8-2% of the total aflatoxinconcentration in dry matter, butcan be as high as 6% in highproducing cows. Also, Stroud (2006) reported that 50 µg kg^{-1} of AFB_1 concentrations in cows feed is sufficient to exceed the FDA action limit of 0.5 µg/L AFM_1 in milk, assuming transfer of 1.0% into milk. Maximum levels of AF in milkregulated in many countries, which the maximum level estimated with0.05 µg kg^{-1} in EU standard and 0.5 µg kg^{-1} in USA according to FDA regulation. It is obvious that the AFM_1 concentration in the milk of control group met the European standard (0.05 µg/kg milk) while the concentration of AFM_1 in milk of the group fed contaminated corn silage was very high which exceeded from 6-12 times the concentration in milk of control group.

Table (6). Effect of experimental treatmentson DMI, average milk production, milk constituentsandaflatoxin M_1in milk.

Item	Control TMR	Contaminated TMR	p-value
Number of animal (N)	12	12	
DMI , kg h^{-1} d^{-1}	23.9±0.03[a]	20.6±0.21[b]	0.042
Milk yield, kgh^{-1} d^{-1}	31.57±0.28[a]	24.73±0.18[b]	0.0001
Fat corrected milk yield, kg h^{-1} d^{-1}	30.244	22.84	0.0001
Milk fat, %	3.72±0.09	3.49±0.06	0.55
Milk protein, %	2.94±0.03	2.81±0.03	0.39
Milk lactose, %	4.07±0.04[a]	3.70±0.03[b]	0.045
Milk total solids, %	10.74±0.08	10.81±0.07	0.85
Milk urea, mg kg^{-1}	19.28±0.67	25.625±0.61	0.056
Somatic cell count ($X10^3$ cells/mL)	101.833±8.46	154.667±10.44	0.27
Feed conversion ratio, kg / kg milk			
Kg DMI/ kg milk	0.76	0.83	
Kg DMI/ kg FCM	0.79	0.90	
Aflatoxin M1 concentration in the milk, µg kg^{-1}			
1st week $AFM1^1$,µg kg^{-1}	0.035	0.280	
2nd week $AFM1^1$,µg kg^{-1}	0.055	0.320	
3rd week $AFM1^1$,µg kg^{-1}	0.045	0.320	
4th week $AFM1^1$,µg kg^{-1}	0.030	0.360	

Values in a row followed by a different superscripts are significantly different (P<0.05).

CONCLUSION

The present results support that the hygienic quality of silage is more important than using mycotoxins deactivator for animal performance, even with using low percent of contaminated feed and maintain the level of total aflatoxin and aflatoxin B_1concentration lower than the permissible limits.Feeds naturally contaminated with harmful mycotoxinseven below the FDA limitation (for aflatoxin) in the feed diet canadversely affect the feed intake and nutrients digestibility,rumen fermentation,milk production of dairy cows and increase aflatoxin M_1transferring rate into the milk.

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تأثير استخدام مضادات السموم مع سيلاج الذرة الملوث طبيعيا أوغيرالملوث بالسموم الفطريةعلىأداء الأبقار الحلابة.

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السموم الفطرية و خاصة افلاتوكسين ب1 و الزيرالينون تسبب العديد من التأثيرات الضارة في الحيوانات المزرعية. لا تقف مشكلة السموم الفطرية عند حد التأثير الضار على الحيوان و لكنها تتركز في اللبن و يمكن ان يصل تأثيرها للإنسان. و لهذا تهدف هذه الدراسة الى تقييم تأثير استخدام مضادات السموم في علائق الحيوانات الحلابة لتقليل الأثر الضار على أداء الحيوان و أمان الالبان. استخدم في الدراسة 24 بقرة هولشتين حلابة في الموسم الأول و المواسم التالية قسمت على مجموعتين تحتوي كل منها على 12 بقرة و استمرت التجربة لمدة شهر. غذيت الحيوانات على علائق كلية مخلوطة بكميات تكفي و وتزيد بمقدار 10% عن حاجة الحيوانات لتوفير التغذية المفتوحة. غذيت المجموعة الضابطة (المقارنة) على علائق كلية مخلوطة تحتوي على 10 % سيلاج أذرة عالي الجودة الميكروبية + مضادات السموم الفطرية بينما غذيت المجموعة المعاملة على علائق كلية مخلوطة تحتوي على 10 % سيلاج أذرة منخفض الجودة الميكروبية (ملوث طبيعيا بالسموم الفطرية) + مضادات السموم الفطرية. لوحظ انخفاض معنوي في العدد الكلي لبكتريا الكرش الحية و كذلك عدد خلايا البكتريا المحلله للسليلوز في المجموعة المعاملة بالنسبة للمجموعة المقارنة. أيضا ادت التغذية على السيلاج الملوث الى انخفاض تركيز الأسيتات و البروبيونات و لم يكن لمضادات السموم دور في ايقاف التأثير الضار. أيضا انخفض معامل هضم المادة الجافة و الألياف الخام و المواد الكربوهيدراتية غير الليفية بالمقارنة بالمجموعة المقارنة بينما لم يلاحظ فروق معنوية في معامل هضم المادة العضوية و البروتين الخام و الدهن الخام. أيضا لم يلاحظ فروق معنوية في تركيز البروتينات الكلية و جلوبيولين الدم و النسبة بين الألبيومين و الجلوبيولين و تركيز البيلروبين و كذلك نشاط انزيمات الكبد . سجلت الأبقار المغذاه على السيلاج الملوث انخفاض في كمية المأكول و متوسط انتاج اللبن و و زيادة تركيز افلاتوكسين M1 في اللبن. و هذه النتائج تشير إلى أن جودة و سلامة السيلاج اكثر اهمية من استخدام مضادات السموم الفطرية و حتى و ان تم استخدام نسب قليلة من العلف الملوث و كانت نسبة السموم الفطرية في الحدود المسموحة.