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Effect of Esterified Glucomannan on Carryover of Aflatoxin from Feed to Milk in Lactating Holstein Dairy Cows

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Authors' contributions

This work was carried out in collaboration between all authors and all authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the effect of different levels of esterified glucomannan (EG) on detoxification and carryover of aflatoxin (AF) from feed to milk in lactating Holstein dairy cows.
Study Design: The experiment was designed as a randomized block with twelve cows allocated to each treatment group.
Place and Duration of Study: Department of Animal Science, Faroogh Life Sciences Research Laboratory, between July 2011 and August 2012.
Methodology: Forty-eight lactating Holstein dairy cows were individually fed a similar based ration and randomly allocated to one of four levels of EG as the experimental treatments (0, 18, 27 and 36 g/cow daily of EG, named EG-0, EG-18, EG-27 and EG-36, respectively). Milk samples were collected on d 20 and 21 of experimental period to evaluate changes in milk AF concentration, milk AF secretion (milk AF concentration × milk yield); and AF transfer from feed to milk (AF secretion as a percentage of AF intake).
Results: Feed intake and milk production were not affected by dietary treatments (P>0.05) and averaged 22.08 kg and 37.57 kg/d, respectively. Milk composition was also not affected (P>0.05) by addition of EG in the diet. Inclusion of EG to the diet was not effective in reducing milk aflatoxin M1 (AFM1) concentrations (P>0.05) and averaged 35, 40, 51 and

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38 ng/kg for the EG-0, EG-18, EG-27 and EG-36, respectively. In addition, there was no significant difference (P>0.05) between the dietary treatments regarding AFM1 excretion and transfer of AF from feed to milk. Transfer of AF from feed to milk averaged 1.3, 1.47, 1.86 and 1.24% for the EG-0, EG-18, EG-27 and EG-36 treatments, respectively.
Conclusion: Inclusion of EG up to 36 g/d (3 time more than recommended dosage) was not effective in reducing AFM1 concentrations, AF excretion, or AF transfer from feed to milk.

Keywords: Esterified glucomannan; milk; aflatoxin; lactating cow.

1. INTRODUCTION

Aflatoxins (AF) are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB1), the most abundant AF in naturally contaminated foods and feeds, is toxic and carcinogenic to humans and animals. In ruminants, toxic effects are associated with liver damage, decreased growth efficiency, diminished milk production and quality, impaired resistance to infectious diseases, and impaired vaccine induced immunity [1]. Feeding aflatoxin-contaminated diets to lactating animals results in secretion of aflatoxin M1 (AFM1) in the milk [2]. Both AFB1 and AFM1 are classified as group 2B human carcinogens by the International Agency for Research on Cancer. The toxicity and carcinogenicity of AFM1 is less than AFB1; however, it remains a significant contaminant of concern for food products [3]. The transfer of AF from feed to milk is of critical concern because it is regulated in most countries. In the United States, the Food and Drug Administration (FDA) has set an action level of 0.5 ppb for AFM1 in milk. Milk containing AFM1 concentrations above the critical level must be discarded, causing significant economic loss for the dairy producer. Similar regulations exist in Iran that the action levels have been officially set at 0.1 ppb for AFM1 in milk [4].

Various physical, chemical and biological approaches have been proposed to detoxify AF contaminated feed and feedstuffs [5] but are not extensively applied in practice due to their cost and limited efficacy [6]. One approach to reduce the incidence of aflatoxicosis in farm animals is the use of feed additives that restrict the bioavailability of toxins. A number of studies have shown that sequestering agents such as montmorillonite clays (a product often characterized as hydrated sodium calcium aluminsilicate (HSCAS)) effectively bind AFB1 *in vivo* and protect animals from the effects of dietary AFB1 and to prevent or reduce AFM1 secretion into milk [7,8,9,10]. However, these compounds are relatively inefficient toward others mycotoxins [11].

In recent years, results of some studies have shown that using a natural organic product, esterified glucomannan (EG) a cell wall derivative of *Sacchromyces cerevisiae*, is beneficial as a low-inclusion toxin-binder in minimizing the adverse effects of AF present in contaminated livestock and poultry feeds [12,13,14]. However, results of some studies showed that EG was not effective in reducing milk AFM1 concentrations in dairy cows [8,9, 10]. The objective of the present study was to determine the effect of different levels of EG on detoxification and carryover of AF from feed to milk in lactating Holstein dairy cows fed a similar ration.

2. MATERIALS AND METHODS

Forty-eight lactating Holstein cows [595 ± 61.2 kg BW; 95 ± 17 days in milk; 34.7 ± 4.8 kg milk yield] were randomly allocated to one of four treatments (n=12) being:1) basal diet without EG (EG-0) , 2) basal diet + 18 g/day EG (EG-18), 3) basal diet + 27 g/day EG (EG-27) and 4) basal diet + 36 g/day EG (EG-36). Ingredients and nutrient composition of the diet is shown in Table 1 and were formulated to meet the nutrient requirements for the group average milk production [15]. Cows were housed individually in tie stall barn, provided ad libitum access to feed and water, fed twice daily at 08:00 and 16:00 h, and milked three times daily at 05:00, 14:00 and 22:00 h. The daily dose of EG was divided into 2 aliquots and each aliquot was mixed with 300 g of the concentrate. At each feeding, cows were first fed the aliquots of EG in the concentrate mix and then fed half of their daily aliquot of total mixed ration (TMR). Diets were fed as a TMR for approximately 50 g/kg refusals. Animals were cared for according to the Iranian Council of Animal Care guidelines.

Table 1. Ingredient and chemical composition of the total mixed ration fed to all lactating dairy cows

Item	Amount (% of diet DM)
Ingredients	
Alfalfa hay	25.4
Corn silage	18.3
Barley grain, rolled	18.3
Corn grain, grind	11.4
Cotton seed meal	4.5
Cotton seed	8.1
Soybean meal	7.9
Wheat bran	4.2
Calcium carbonate	0.6
Sodium bicarbonate	0.5
Mineral-vitamin premix[b]	0.8
Chemical composition	
DM	46.7
Crude protein	16.5
Net energy for lactation (Mcal/Kg)	1.54
Neutral detergent fiber	34.9
Acid detergent fiber	22.4

[b] *Guaranteed analysis: 190 g/kg Ca, 90 g/kg P, 50 g/kg Na, 20 g/kg Mg, 50 g/kg K, 3 g/kg Zn, 2 g/kg Mn, 3 g/kg Fe, 0.3 g/kg Cu, 0.001 g/kg Se, 0.1 g/kg Co, 0.1 g/kg I, 500 IU/g of vitamin A, 100 IU/g of vitamin D, and 1 IU/g of vitamin E.*

The experiment was designed as a randomized block with twelve cows allocated to each treatment group, blocked by cow parity (1or >1). The experimental period consisted of 14 days of acclimation followed by 7 days of data and sample collection.

Feeds and orts were weighed daily from d 15 to 21 to determine the feed intake of cows by difference. Feed samples were collected daily to determine dry matter (DM), crude protein (CP) [16], NDF, ADF [17] and TMR contamination with AFB1. Diet DM contained 4.6 ppb of AFB1.

Milk production was recorded on d 20 and 21. On each of the two days, milk samples from the three milkings were composited and frozen until analyzed. Milk samples were analyzed for milk composition (fat, protein, lactose and Solid nonfat) by an automated milkoscan (Foss Electric, Denmark).

Milk samples were analyzed for AFM1 using HPLC (Waters Breeze 1525 HPLC Pump) and affinity columns (reverse phase ODS - 5 µm, 4.6 m×250 m C18 Column TSK-GEL®) based on the method applied to the determination of AFM1 in raw liquid milk [16]. Samples were defatted prior to analysis. A 35 ml sample of defatted milk was then passed through the AFM1 immunoaffinity cleanup columns and filtered. Water was passed through the column and AFM1 eluted and collected in a scintillation vial. The eluate was analyzed by HPLC. Concentrations of AFM1 were determined relative to a quantitative AFM1 standard.

The efficacy of different levels of EG for binding of AF was evaluated based on three measures of effectiveness. These measures include the reduction in milk AF concentration, the reduction in milk AF secretion (calculated as milk AF concentration × milk yield), and reduction in AF transfer (calculated as AF secretion as a percentage of AF intake).

Data were analyzed as a randomized block design using the MIXED procedure [18] according to the following model: $Y_{ijk} = \mu + T_i + B_j + C_k + e_{ijk}$, where Y_{ijkl} is the dependent variable, μ is the overall mean, T_i is the fixed effect of treatment (i =1 to 4), B_j is the fixed effect of block (j =1 to 2), C_k is the random effect of cow (k =1 to 4) and e_{ijk} is the residual error. The significance of differences among treatments was tested using Duncan's multiple range tests, and statistical significance was declared at $P<0.05$.

3. RESULTS AND DISCUSSION

Feed intake and milk production were not affected by the treatments ($P>0.05$) and averaged 22.08 kg and 37.57 kg/d (Table 2), respectively, across all treatments. Milk composition (Table 2) was also not affected ($P>0.05$) by the addition of EG to the basal diet; with fat percentage, protein percentage, lactose percentage and solid nonfat percentage averaging 3.7%, 3.1%, 4.7% and 8.6%, respectively, across all treatments. Information regarding the effect of EG or similar compounds on milk production is scare. Our results confirmed the finding of Bagheri et al. [19] who reported that inclusion of 32 g/cow daily of yeast cell wall product (EG), had no significant effect on feed intake, milk yield and composition of lactating cows. In other hands Nocek et al. [20] reported a significant increase in milk protein percentage when they compared cows fed an enzymatically hydrolyzed yeast product (a nonlive product that may be comparable to EG) to those fed yeast culture or no additive.

The concentrations of AF residues in milk are summarized in Table 3. Aflatoxin M1 concentrations of the milk secreted for the animals fed EG-0, EG-18, EG-27 and EG-36, averaged 35, 40, 51 and 38ng/kg, respectively. Compared with the EG-0 treatment, the addition of EG to the basal diet was not effective in reducing milk AFM1 concentrations ($P>0.05$). Aflatoxin M1 excretion via milk, as calculated from milk AFMI concentration and total milk volume produced, was 1323, 1492, 1918, and 1241 ng/d for EG-0, EG-18, EG-27 and EG-36, respectively (Table 3). Besides, the addition of EG to the basal diet was not effective in reducing AFM1 excretion in milk ($P>0.05$). Transfer of AF from feed to milk, as calculated from AF intake and total milk volume, averaged 1.3, 1.47, 1.86 and 1.24% for the EG-0, EG-18, EG-27 and EG-36 treatments, respectively. Compared with EG-0, the addition of EG to the basal diet was not effective in reducing AF transfer from feed into milk ($P>0.05$).

Table 2. Dry matter intake, milk yield and composition of Holstein lactating cows fed total mixed ration containing different levels of esterified glucomannan as 0(EG-0), 18(EG-18), 27(EG-27) or 36(EG-36) g/cow/day

Item	Treatment					
	EG-0	EG-18	EG-27	EG-36	SEM	*P*-value
DMI	22.14	22.10	22.42	21.67	0.41	0.41
Milk (kg)	37.8	37.3	37.6	37.6	0.92	0.7
Fat (%)	3.64	4.08	3.61	3.64	0.076	0.25
Protein (%)	3.18	3.12	3.09	3.14	0.015	0.19
Lactose (%)	4.74	4.66	4.61	4.69	0.022	0.19
Solid nonfat (%)	8.69	8.53	8.46	8.58	0.04	0.21

Table 3. Milk aflatoxin M1 (AFM1) concentration, milk aflatoxin excretion, and milk aflatoxin transfer from feed to milk of Holstein lactating cows fed total mixed ration containing different levels of esterified glucomannan as 0(EG-0), 18(EG-18), 27(EG-27) or 36(EG-36) g/cow/day

Item	Treatment					
	EG-0	EG-18	EG-27	EG-36	SEM	*P*-value
AFM1 (ng/kg)	35	40	51	38	8.4	0.28
Excretion (ng/d)	1323	1492	1918	1241	438	0.31
Transfer (%)	1.3	1.47	1.86	1.24	0.37	0.37

AFM1 excretion = concentration of AFM1 in milk × amount of milk produced; aflatoxin transfer = excretion of AFM1 divided by aflatoxin B1 (AFB1) consumption (× 100).

Milk AFM1 concentrations ranged from 35 to 51ng/L, and transfer rates of AF from feed to milk ranged from 1.24 to 1.86%. Transfer rates observed in the current study are consistent with previous reports for dairy cows indicating AF transference rates ranging from 0.25 to 4.8% [8,9,21,22,23].

Results of this study indicated that inclusion of EG up to 36 g/d (3 time more than recommended dosage) was not effective in reducing AFM1 concentrations, AF excretion, or AF transfer from feed to milk.

Our results confirmed previous results [8,9,10], who reported that MTB-100 (another commercial modified yeast cell culture preparation based on a Saccharomyces cerevisiae) was not effective in reducing milk AFM1 concentrations, AF excretion, or AF transfer from feed to milk in lactating dairy cow. Stroud [8], Kuts et al. [9] and Kissell et al. [10] used 115, 125 and 10 g/cow daily of MTB-100 in the lactating dairy cow, respectively. Results of these studies were in contrast to finding of Diaz et al. [7] in which MTB-100 at 0.05% of a diet DM was reported to reduce milk AFM1 concentrations by 59%.

The differences in milk AFM1 reduction among various feed additives might be due to their composition and mechanism of action of the active compounds. Montmorillonite clays are sources of HSCAS, whereas EG contains a modified yeast cell culture. Phillips et al. [24] proposed that AF was bound to HSCAS as a result of the β-carbonyl system of AF forming a complex with uncoordinated edge site aluminum ions of HSCAS. It is now postulated that the major site of chemisorption of AF to HSCAS is at the interlayer surfaces [25]. The dicarbonyl portion of the AF molecule was found to be essential for tight binding of the molecule by

HSCAS. The interaction with HSCAS makes AF unavailable for absorption. In contrast, the mechanism for the modified yeast product has not been well described yet.

4. CONCLUSION

Present results demonstrated that the inclusion of different levels of EG up to 36 g/cow daily; resulted in no changes in feed intake, milk yield and composition of lactating Holstein cows. In addition, EG concentrations as used in the present study was not effective in reducing milk AFM1 concentrations, AF excretion or AF transfer from feed to milk under the conditions of this experiment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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