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Effectiveness of Magnetic Bentonite Nanocomposites as Mycotoxin Binders in Dairy Baluchi Ewe's Diets: Impact on Milk Yield, Composition, Blood Chemistry, and Aflatoxin M1 Levels

Research Article

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ABSTRACT

Bentonite is considered the most effective adsorbent for aflatoxin (AF) decontamination, and recent studies have shown that changing its structure in nano form improves its physicochemical properties and chemical stability. This study was aimed to evaluate the effectiveness of different types of bentonites as binders on performance, plasma metabolites, and aflatoxin M1 (AFM1) levels in contaminated milk of Baluchi ewes. The study was conducted with 12 ewes randomly assigned to four different experimental diets. The experimental diets were: (1) control (the basal diet had no supplements and contained bakery waste naturally contaminated with AF); (2) control diet supplemented with natural bentonite (NB) (5 g/kg DM); (3) control diet supplemented with modified bentonite (MB) (5 g/kg DM) and (4) control diet supplemented with magnetic bentonite nanocomposite (MBNC) (5 g/kg DM). The study found that adding bentonite clays to the diet of ewes resulted in increased milk yield (P<0.01) and milk components (P<0.01) such as fat, protein, lactose, total solids, and solids not fat. The highest milk yield and milk components were observed in the MBNC treatment. However, there was no significant difference in glucose, urea, cholesterol, albumin, globulin, and total protein among the diets (P>0.05). The study also found that increasing aflatoxin B1 (AFB1) intake resulted in a decreased carryover of AFB1 into AFM1 (P<0.01), with MBNC having the lowest carryover compared to other treatments (P<0.01). These results suggest that modification of bentonite structure in nanocomposite form improves chemical stability, physicochemical properties, and efficiency as novel toxin binders for crops and animal products.

KEY WORDS bentonite, milk, mycotoxin, nanocomposites, plasma metabolites, toxin binders.

INTRODUCTION

Aflatoxin (AF) is predominantly synthesized by *Aspergillus flavus* and *Aspergillus parasiticus* (Awuchi *et al.* 2021; Hamad *et al.* 2023b). Currently, researchers have identified several types of aflatoxins, but the most commonly identified are aflatoxins B1, B2, G1, and G2 (Abdin *et al.* 2010; Benkerroum, 2020). Various approaches, such as physical, biological, and chemical assays, are employed to detoxify aflatoxin in crops and livestock products. From the point of view of prior studies, chemically inert bentonite clays are considered the most effective adsorbents for decontaminating aflatoxin (Upadhaya *et al.* 2010; Nones *et al.* 2017).

Bentonite ($(Na,Ca)(Al,Mg)(Si_4O_{10})3(OH)_{6n}H_2O$) consisting of montmorillonite as a significant component, possesses colloidal properties attributed to its aluminosilicate structure (Maryan and Montazer, 2015; Tate *et al.* 2015). The presence of bentonite, with its prominent physiochemical features (i.e., surface specificity, enlargement, adsorption, cation exchange, low cost, high safety, and colloidal properties), can lead to the removal of organic matter (Nones *et al*. 2017).

Studies have shown that bentonite clays can decrease levels of AF in contaminated milk (Agag, 2003). Hamad *et al*. (2022) determined that calcium and sodium bentonites have the potential to function as novel food-safe adsorbents for ochratoxin A (OTA) in cheese samples. However, the mycotoxin adsorbents mentioned above often bind to other minerals and vitamins in the diet, making them inactive (Swain *et al*. 2016). Although conventional methods are constantly improving, recent research findings are looking for innovative solutions. By altering bentonite's structure via different methods, the performance of bentonite can be optimized. Eliminating phosphate types via a novel bentonite-alum absorptive suggested by Mahadevan *et al*. (2018). Huang *et al*. (2017) could synthesize the organo-bentonite using cetyl trimethyl ammonium bromide (CTAB) in the bentonite's structure. Martinez *et al*. (2017) demonstrated the ability of the modified bentonite alum polymer to coat ceramic substrates. Recent advances in the production and applications of bentonite nanocomposite to remove inorganic materials from water were reviewed by Pandey (2017). The impact of nano bentonite synthesis on *Salmonella typhimurium* mutation was investigated by Degtyareva *et al*. (2016). Bama and Sundrarajan (2017) produced an antibacterial Ag/TiO2/bentonite nanocomposite against some bacterial species.

According to recent studies, modifying the structure of bentonite to nano and nanocomposite improved its chemical stability and physiochemical properties (El-Nile *et al*. 2021). At the same time, assessing the detoxification capacity of bentonite in reducing aflatoxin M1 (AFM1) levels in raw milk is a widely used method (Hamad *et al*. 2023a). So, we anticipate that due to the new physiochemical properties of bentonite nanocomposite, the chemical aflatoxin decontamination of crops and livestock products will be managed effectively. Therefore, the aim of this study was to evaluate the effectiveness of different types of bentonite as binders on animal performance, plasma metabolites, and AFM1 levels in the milk of Baluchi ewe's that were fed on aflatoxin-contaminated diets.

MATERIALS AND METHODS

Experimental feed additives

Natural aluminosilicate structured bentonite (Bentofeed™) and modified bentonite (Zarin Binder^{plus} ™) were supplied from Vivan Trading Company, Qaen, Iran. The chemical composition and physical properties of the aluminosilicate structured bentonite used in the study are presented in Tables 1 and 2. To synthesize the $Fe_3O_4^-$ bentonite nanocomposite, we vigorously stirred 50 mL of distilled water at 80 ˚C and dissolved 2 g of $FeCl_2.4H_2O$ and 5.2 g of $FeCl_3.6H_2O$. Subsequently, 200 mL of 25% NH_4OH was gradually added to the mixture. Then, three g of bentonite was added to the mix. After three hours, a magnet was used to isolate the magnetic bentonite nanocomposite particles. Synthesized bentonite nanocomposite particles were washed via ultrapure water and dried at 50 ˚C for 24 hours (Heydari *et al*. 2019). To detect the surface morphology of the adsorbent, the scanning electron microscopy (SEM) figures (SEM, TESCAN Mira3) and Energy Dispersive X-ray (EDX) spectrum were used (Figures 1 and 2). Fourier transformed infrared (FTIR) spectrum of a $Fe_3O_4^-$ bentonite nanocomposites particles was obtained from 400 to 4000 cm^{-1} (Perkin Elmer 1750 FTIR Spectrophotometer) (Figure 3) (Sulaymon *et al*. 2014; Heydari *et al*. 2019).

Animals, diet, and treatments

The study was conducted at the Torbat-e Jam animal husbandry farm in Iran. The animals were kept according to the Iranian Council on Animal Care (1995). Twelve Baluchi ewes [55 ± 2.5 kg body weight (BW), first-parity and early lactation] were randomly assigned to four different experimental diets (n=3 per group) using a completely randomized design.

Experimental diets were: (1) control (the basal diet had no supplements and contained bakery waste naturally contaminated with AF); (2) control diet supplemented with natural bentonite (NB) (5 g/kg DM); (3) control diet supplemented with modified bentonite (MB) (5 g/kg DM); and (4) control diet supplemented with magnetic bentonite nanocomposite (MBNC) (5 g/kg DM). The diets were formulated following the NRC (2007) guidelines (50:50 forage-to-concentrate ratio). Ewes were given time to adapt to the experimental diets for seven days. After the initial period, the trial continued for a further 21 days. The ewes were fed with an unlimited supply of feed (5% refusals) twice daily (7 a.m. and 7 p.m.), had free access to fresh water, and were milked twice a day (6 a.m. and 6 p.m.).

Animal sampling

Daily dry matter intake (DMI), orts, and milk yield were measured for ewes during the trial. The samples of the diets were dried in a forced-air oven at 65 ˚C for 48 hours and then placed in plastic bags for chemical analysis. Blood samples were collected at weeks 4 (5 consecutive days), three hours after the morning feeding, by using heparin tubes through the jugular vein (5-10 mL). The samples were then centrifuged (3000×g, 10 min), and the resulting plasma supernatant was drawn into sterile 1.5 mL microtubes and stored at -80 ˚C for subsequent analysis.

Table 1 Chemical composition and physical properties of the natural and modified forms of bentonite

Item	Natural bentonite (Bentofeed™)	Modified bentonite (Zarin Binder[plus ™])
Chemical composition (%)		
TiO_2	0.22	0.22
CaO	2.65	2.65
K_2O	0.75	0.75
Na_2O	2.67	2.67
MgO	2.57	2.57
Fe_2O_3	2.34	2.34
Al_2O_3	12.7	12.7
SiO_2	64.5	64.5
Physical properties		
Water absorption capacity (%)	700-750	700-750
Swelling index (ml/2g)	19-21	19-21
Moisture content (%)	4-8	4-8
Particle size (mesh)	50-400	37
CEC (meq/100g)	100-110	100-110
Heavy metals (ppm)		
Pb	13	11
Cd	0.1	0.1
Hg	< 0.05	< 0.05
Ni	-	7
As	2.43	3
Microbial analysis (10^4/g)		
Added microbial population	-	1.80×10^2

Table 2 Major ingredients and chemical composition of the experimental basal diet based on dry matter (DM)

Alfalfa hay	35.0
Wheat straw	15.0
Barley grain	6.00
Corn grain	17.0
Wheat bran	12.5
Naturally contaminated bakery waste	7.50
Soybean meal	4.00
Vitamins and minerals mixture[1]	3.00
Chemical composition (% DM)	
Dry matter	90.03
Crude protein (%)	12.99
Neutral detergent fiber (%)	35.00
Ether extract (%)	03.61
Ash (%)	09.65
Metabolizable energy (Mcal/kg DM)	02.41
Aflatoxin concentration (µg/kg)	
Aflatoxin B1	04.21

[1] Mineral and vitamin mixture (mg/kg): vitamin E: 100 mg; vitamin B_1: 10 mg; vitamin B_2: 20 mg; vitamin A: 400000 IU; vitamin D: 100000 IU; Ca: 30 g; P: 12 g; Na: 40 g; Cu: 1000 mg; I: 60 mg; Co: 60 mg; Mn: 2000 mg; Zn: 2000 mg; Fe: 3000 mg.

Milk samples were collected from ewes during last week of the trail for five consecutive days. The milk yield was mixed, and a portion of it was analyzed for chemical composition and AFM1 residues.

Analytical procedures

The AOAC protocol (AOAC, 2005) was used to determine the dry matter (method no. 930.15), ether extract (EE, method no. 991.36), ash (method no. 942.05), and crude protein (CP, Kjeldahl, N×6.25, method no. 954.01) concentrations.

The procedure outlined by Van Soest *et al.* (1991) and the protocol of Ankom Technology (2006) were followed during measuring neutral detergent fiber (NDF) via the Ankom fiber analyzer (ANKOM, model A2001, New York, USA). Milk components such as protein, fat, lactose, total solids (TS), and solids not fat (SNF) were assessed through the employment of a milkoscan analyzer (Foss Electric, Conveyor 4000, Hillerød, Denmark). The levels of glucose, urea, cholesterol, albumin, globulin and total protein were analyzed using an auto-analyzer (Biosystems A15; 08030 Barcelona, Spain).

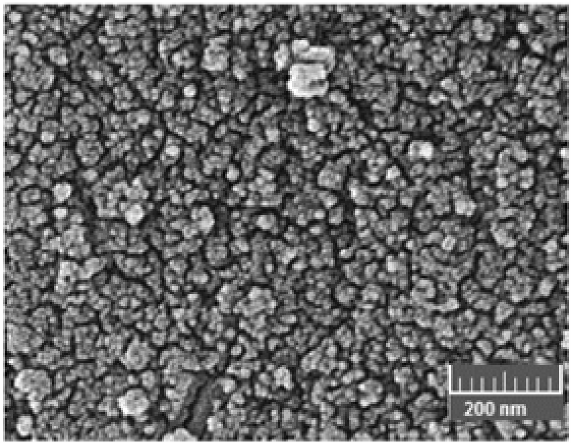

Figure 1 Scanning electron microscopy image of magnetic $Fe_3O_4^-$ bentonite nanoparticles on a 200 nm scale

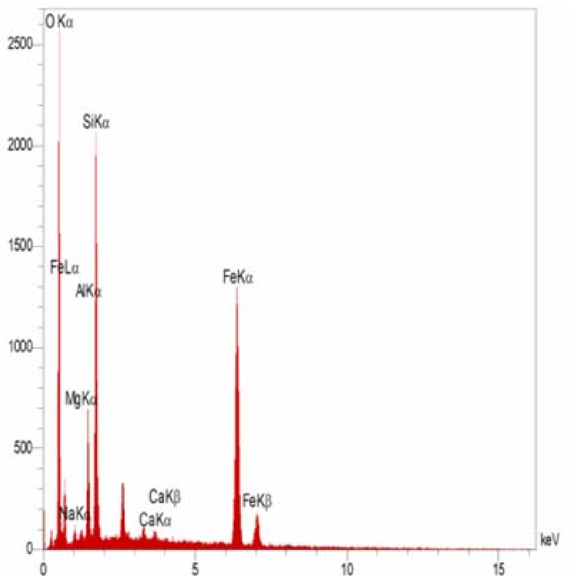

Figure 2 Energy Dispersive X-ray spectrum obtained for magnetic $Fe_3O_4^-$ bentonite nanoparticles

A high-performance liquid chromatography (HPLC) device [(SHIMADZU, Japan), (solid phase: C18 (50 mm×4.6×5 µm), mobile phase: acetonitrile/phosphate buffer solution, excitation wavelength: 365 nm, emission wavelength: 435 nm, detector: SHIMADZU HPLC fluorescence detector (RF-X10A), mobile phase flow: 2-3 mL/min] was used to separate and quantify the concentration of aflatoxin. The carryover of AFM1 in milk was calculated as the ratio between the AFM1 excreted in milk and the intake of AFB1 (Battacone *et al.* 2009).

Statistical analysis

All data were analyzed via PROC GLM of SAS (2004) with the following model:

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

Where:
Y_{ij}: value of each observation.
µ: overall mean.
T_i: treatment effect.
e_{ij}: experimental error.

Duncan's multiple range test measured the treatment's statistical difference (P<0.05).

RESULTS AND DISCUSSION

The impact of NB, MB, and MBNC supplementation on DMI, lactation and milk components is exhibited in Table 3. The addition of bentonite clays increased DMI, milk yield and milk (6% fat–corrected Milk) (P<0.01) (Table 3). Compared with the control, NB, and MB, the highest DMI and milk yield was observed in MBNC. The inclusion of bentonite clays resulted in a significant increase (P<0.01) in milk components such as fat, protein, lactose, TS, and SNF (% and g/d), except for protein percentage (Table 3). Similarly, the milk components were highest for MBNC compared to other treatments.

We observed that the addition of bentonite clays increased DMI, milk yield and fat-corrected milk (6% FCM) in Baluchi ewes. The results of our study support the findings of Walz *et al.* (1998) and Kazemi *et al.* (2017), who suggested that the addition of bentonite clays leads to an increase in DMI. Increased dietary DMI led to an increase in milk production or FCM in ewes (Kazemi *et al.* 2017). An increase in the transfer of microbial protein to the small intestine has been observed with the use of bentonite clay (Ivan *et al.* 1992). By increasing the flow of nutrients from the rumen to the small intestine, an increase in the productivity of ruminants is expected, especially with regard to meat and milk production (Kazemi *et al.* 2017). In the current study, higher milk yield could be associated with better ration quality and increased nutrient delivery to the small intestine after bentonite clay consumption. Hamad *et al.* (2023a) reported that the milk composition of treated groups with bentonite clays (HAFR 3 and HAFR 4) was significantly increased in fat, protein, and SNF compared with the control. Kholif *et al.* (2015) and Morsy *et al.* (2016) found that the use of bentonite and montmorillonite treatments resulted in increased lactose and milk energy contents due to improved nutrient digestibility and optimized ruminal fermentation. In our experiment, the increase in milk components (% or g/day) in treated animals compared to the control group was due to the reduction in mycotoxin side effects (Moschini *et al.* 2008).

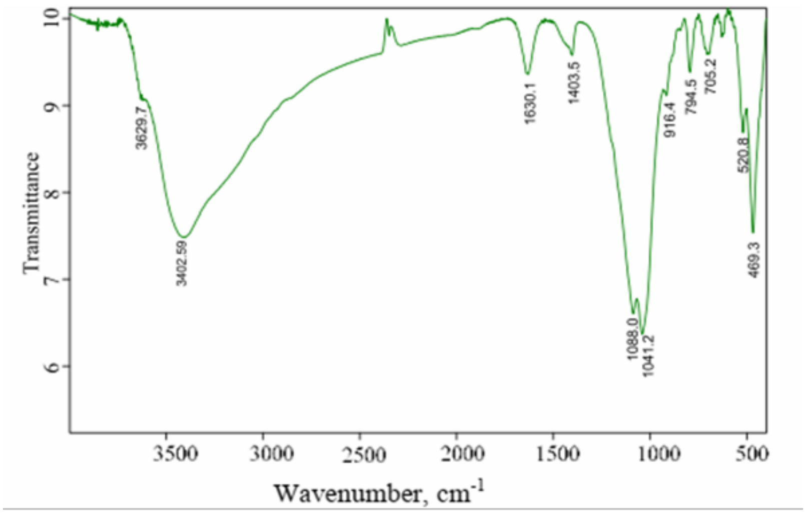

Figure 3 Fourier transformed infrared (FTIR) spectrum of magnetic $Fe_3O_4^-$ bentonite nanoparticles

Table 3 Effects of basal diet supplementation with different toxin binders on lactation and milk components of Baluchi ewes

Items	Treatments[1]				SEM	P-value
	Control	NB	MB	MBNC		
DM intake (g/d)	1757.2[a]	1858.1[b]	1862.7[b]	1937.0[c]	13.24	< 0.001
Yield						
Milk (g/d)	856.25[a]	955.75[b]	960.00[b]	993.5[c]	13.69	< 0.001
Milk (6% FCM, kg/d)[2]	1.03[a]	1.10[b]	1.10[b]	1.10[b]	0.009	< 0.001
Milk components (%)						
Fat	7.36[a]	7.69[b]	7.73[b]	8.34[c]	0.09	< 0.001
Protein	4.56	4.42	4.60	4.70	0.05	0.35
Lactose	4.65[a]	4.77[a]	4.76[a]	5.08[b]	0.04	< 0.001
Total solids	16.5[a]	16.9[a]	17.0[a]	18.1[b]	0.16	0.002
Solids not fat	9.21[a]	9.19[a]	9.36[a]	9.78[b]	0.80	0.01
Milk components (g/day)						
Fat	63.1[a]	73.5[b]	74.1[b]	82.8[c]	1.86	< 0.001
Protein	39.1[a]	42.4[ab]	44.0[bc]	46.7[c]	0.91	0.009
Lactose	39.9[a]	45.7[b]	45.4[b]	50.4[c]	1.00	< 0.001
Total solids	142.2[a]	162.4[b]	163.1[b]	180.1[c]	3.65	< 0.001
Solids not fat	79.0[a]	88.2[b]	89.5[b]	97.2[c]	1.82	< 0.001

[1] Control: the basal diet had no supplements and contained bakery waste naturally contaminated with AF; NB: natural bentonite, control diet supplemented with natural bentonite, 5 g/kg DM; MB: modified bentonite, control diet supplemented with modified bentonite, 5 g/kg DM basal diet and MBNC: magnetic bentonite nanocomposites, control diet supplemented with magnetic bentonite nanocomposite, 5 g/kg DM basal diet.
[2] Fat-corrected 6% milk (FCM)= M [0.453 + 0.0912f], M: the yield of milk (kg) and f: fat percentage of milk (Mavrogenis and Papachristoforou, 1988).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of basal diet supplementation with different toxin binders on plasma metabolites of Baluchi ewes

Items	Treatments[1]				SEM	P-value
	Control	NB	MB	MBNC		
Glucose (mg/dL)	53.88	58.69	59.85	59.94	1.07	0.13
Urea (mg/dL)	19.34	19.02	19.05	18.73	0.12	0.44
Cholesterol (mg/dL)	55.30	56.91	55.22	55.67	0.64	0.82
Albumin (mg/dL)	02.84	03.15	03.40	03.27	0.10	0.29
Globulin (mg/dL)	03.33	03.13	03.05	03.11	0.08	0.70
Total protein (g/dL)	06.27	06.30	06.47	06.71	0.07	0.14

[1] Control: the basal diet had no supplements and contained bakery waste naturally contaminated with AF; NB: natural bentonite, control diet supplemented with natural bentonite, 5 g/kg DM; MB: modified bentonite, control diet supplemented with modified bentonite, 5 g/kg DM basal diet and MBNC: magnetic bentonite nanocomposites, control diet supplemented with magnetic bentonite nanocomposite, 5 g/kg DM basal diet.

Decontamination of mycotoxins results in better nutrient digestibility and increased microbial protein synthesis, leading to improved milk components (Kholif *et al*. 2014). Bentonite and montmorillonite are considered effective adsorbents for AFB1 due to their high cation exchange capacity (CEC) and specific surface area (El-Kady *et al*. 2009; Vila-Donat *et al*. 2018). Chouikhi *et al*. (2019) and Soltan *et al*. (2021c) found that the nano-bentonite had a high reversible retention capacity compared to the natural forms. This is because of the increased hydrophobic surface, interlayer spacing, and the intercalation of organic cations in nano-montmorillonite. According to Soltan *et al*. (2021c) the absorption efficiency of nano-montmorillonite increased due to its high CEC and increased negative charge. This is why supplemented MBNC is superior compared to the other groups.

Table 4 displays the effect of various bentonite clay supplementations on plasma metabolites. No significant difference was observed in glucose (mg/dL), urea (mg/dL), cholesterol (mg/dL), albumin (mg/dL), globulin (mg/dL), and total protein (g/dL) (P>0.05) among the diets (Table 4). However, NB, MB, and MBNC showed an increasing trend for glucose and total protein compared to the control treatment. Ewes given bentonite clay treatments had lower urea numerically.

In this study, no sign of toxicity was observed until the end of the experiment, and plasma metabolites were within the reference ranges reported by Boyd (1984). Khadem *et al*. (2007) and Kazemi *et al*. (2017) found that sodium bentonite (SB) supplementation did not alter any of the hematological analites or plasma metabolites compared to the control group, which is consistent with the results of this study. Recent research (Gouda *et al*. 2019) has shown that adding clay minerals as a sorbent for mycotoxins in the diet of lactating goats did not affect plasma total protein, albumin, urea, and creatinine. The unaltered plasma metabolites indicate that the nutritional status of ewes given bentonite clays remains unchanged (Hosten, 1990; Gouda *et al*. 2019). In addition, bentonite supplementation improves organic matter (OM) digestibility and positively alters ruminal fermentation (Kholif *et al*. 2016), resulting in increased energy utilization, propionate absorption, and glucose synthesis (Morsy *et al*. 2016). Bentonite application may decrease plasma urea levels by establishing a steady state in the rumen, resulting in increased microbial protein synthesis (Khadem *et al*. 2007). The decrease in urea may be due to H^+ uptake, increased ruminal pH, and increased rumen microbial activity associated with CH_4 reduction and the provision of sufficient ATP as short-chain fatty acids (SCFAs) for more remarkable microbial protein synthesis (Morsy *et al*. 2021; Soltan *et al*. 2021a). Also, an increase in rumen pH can lead to increased protein solubility and affect the synthesis of branched-chain fatty acids (BCFAs) (Apajalahti *et al*. 2019; Ramos *et al*. 2021). So, in the present study, bentonite activity was more efficient in MBNC than other forms and the BCFAs produced can be used for more microbial protein mass (Soltan *et al*. 2021b).

The effects of different experimental diets on the concentration of aflatoxin AFM1 and the carryover of AFB1 into AFM1 in milk are exhibited in Table 5. There was a significant difference in AFB1 intake (µg/d) (P<0.001) between diets. With increasing AFB1 intake, carryover of AFB1 into AFM1 (%) decreased significantly (P<0.001) (Table 5). Compared to control, NB, and MB treatments, MBNC had the lowest carryover of AFB1 into AFM1 (%).

Due to its lipophilic properties and low molecular weight, AFB1 is readily absorbed through the rumen wall and intestines and appears as AFM1 in milk after consumption of contaminated feed (Masoero *et al*. 2007). Clay minerals can adsorb aflatoxins, reducing their availability for gastrointestinal absorption and helping prevent their harmful effects on animals (Ogunade *et al*. 2016). Bentonites are high-quality adsorbent clays made from silicates or aluminosilicates that can absorb up to 100% of their dry water weight and 80% oil (Murray, 2006; Jouany, 2007). Toxins are absorbed into the porous structure of the clay by electrical charges, while the rate of adsorption can be affected by factors such as the size and electrical charge of the toxin or the structure of the clay (Jouany, 2007). According to Queiroz *et al*. (2012), the inclusion of 1% montmorillonite in the cows' diet resulted in a decrease in the AFM1 concentration in their milk. Similarly, Diaz *et al*. (2004) reported that feeding dairy cows with 1.2% SB as an aflatoxin binder reduced milk contamination by up to 61%. The treatments used in this study were within the reference limits for both AFB1 concentration in dairy animal feed concentrates (maximum allowed concentration of 20 µg/kg DM) and AFM1 concentration in milk (limited to 0.05 µg/kg, according to the European Commission, No 1881/2006).

In this study, the feed contained 4.21 g AFB1/kg DM, and bentonite clays reduced the transfer of AFB1 to AFM1 in milk from 0.81% to 0.73%. According to recent studies (Ogunade *et al*. 2016; Maki *et al*. 2016a; Maki *et al*. 2016b; Gan *et al*. 2019), the efficacy of bentonite clays in absorbing toxins is reflected in the decrease in the concentration of AFB1 in the rumen or milk (as AFM1). Bentonite, in its nano form, has a higher surface area than its natural state. This increased surface area provides more active sites for the adsorption of AFM1, making it more efficient in absorbing the toxin. Additionally, the smaller particle size of nano-bentonite allows for better dispersion in feed and easier access to the toxin, leading to more effective toxin binding (Soltan *et al*. 2021c).

Table 5 Effects of basal diet supplementation with different toxin binders on the concentration of aflatoxin M1 (AFM1) and carryover in the milk of Baluchi ewes

Items	Treatments[1]				SEM	P-value
	Control	NB	MB	MBNC		
AFM1 (ng/L)	50.07	50.93	50.16	48.55		
AFB1 intake (μg/d)	7.38[a]	7.82[b]	7.80[b]	8.13[c]	0.05	< 0.001
Carryover[2] %	0.67[a]	0.65[b]	0.64[b]	0.60[c]	0.006	< 0.001

[1] Control: the basal diet had no supplements and contained bakery waste naturally contaminated with AF; NB: natural bentonite, control diet supplemented with natural bentonite, 5 g/kg DM; MB: modified bentonite, control diet supplemented with modified bentonite, 5 g/kg DM basal diet and MBNC: magnetic bentonite nanocomposites, control diet supplemented with magnetic bentonite nanocomposite, 5 g/kg DM basal diet.
[2] Carryover= percentage of aflatoxin B1 that was converted to aflatoxin M1 and excreted in milk (Battacone *et al*. 2009).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This is why the ewes that received supplemented MBNC have lower AFM1 levels in milk than the other groups. These findings support our initial hypothesis that modifying bentonite structure to nanocomposite form improves chemical stability, physicochemical properties, and efficiency as novel toxin binders for crops and livestock products.

CONCLUSION

In conclusion, the addition of bentonite clays, especially MBNC, has a positive impact on milk yield and its components. This is due to reducing mycotoxin side effects, improving nutrient digestibility, and optimizing of rumen fermentation. The increase in milk yield can be attributed to better ration quality and increased nutrient delivery to the small intestine after bentonite clay consumption. In addition, the bentonite supplement has a positive effect on plasma metabolites, improves the digestibility of organic matter, and positively changes rumen fermentation. Using bentonite clays, also, decreases the transfer of AFB1 to AFM1 in milk, making it an effective adsorbent for mycotoxins. The nano form of bentonite absorbs toxins more efficiently due to its larger surface area and smaller particle size. These results suggest that modification of bentonite structure in nanocomposite form improves chemical stability, physicochemical properties, and efficiency as novel toxin binders for crops and animal products.

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