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Research Article

Efficacy of Mycotoxin Adsorbents on Aflatoxin B1 Decontamination and *in vitro* Rumen Fermentation

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Abstract

This study was conducted by using an in vitro gas production technique to study the effect of aflatoxin B1 and mycotoxins adsorbents on aflatoxin B1 decontamination and efficiency of rumen fermentation. There were eight treatments, that assigned into completely Randomized Design: T1=the control group, T2= the diet plus 1 µg/mg of aflatoxin B1 (AFB1), T3, T4, T5 = the diets supplement 0.5% of each adsorbent (activated charcoal, montmorillonite, *Saccharomyces cerevisiae*) and T6, T7, T8 = the diets plus 1 µg/mg of AFB1 together with 0.5% of each mycotoxin adsorbent, respectively. The results were shown that the gas production from the insoluble fraction and the potential extent of gas production in the diet plus the AFB1 were lower than the control ($P<0.05$). Moreover, montmorillonite and *S. cerevisiae* were effectively reduced the negative effect of AFB1 on gas production. In contrast, the diet plus AFB1 and diet plus mycotoxin adsorbents did not interfere the *in vitro* dry matter and organic matter digestibilities, volatile fatty acids and ammonia nitrogen ($P>0.05$). In conclusion, AFB1 had effected and had decreased cumulative gas production. However, montmorillonite and *S. cerevisiae,* as effective adsorbents, could be reduced the adverse effect of AFB1 in this study.

Keywords: Aflatoxin B1, Mycotoxin adsorbent and Rumen fermentation

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ประสิทธิภาพของสารดูดซับพิษจากเชื้อราต่อการขจัดการปนเปื้อนของอะฟลาทอกซิน บี 1 และการหมักในกระเพาะรูเมนในหลอดทดลอง

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บทคัดย่อ

การศึกษาครั้งนี้ได้ดำเนินการโดยใช้เทคนิค *in vitro* gas production technique เพื่อศึกษาผลของอะฟลาทอกซิน บี 1 และสารดูดซับสารพิษจากเชื้อราชนิดต่างๆต่อการลดการปนเปื้อนของสารพิษจากเชื้อราและประสิทธิภาพของการหมัก โดยวางแผนการทดลองแบบสุ่มสมบูรณ์ แบ่งอาหารทดลองออกเป็น 8 ทรีตเมนต์ ประกอบด้วย อาหารกลุ่มควบคุม (T1), อาหารเสริมอะ ฟลาทอกซิน บี 1 เข้มข้น 1 ไมโครกรัม/อาหาร 1 มก. (T2), อาหารเสริมสารดูดซับที่ระดับร้อยละ 0.5 (ผงถ่านกัมมันต์ (T3), มอนต์โมริลโลไนต์ (T4), ยีสต์ *Saccharomyces cerevisiae* (T5), และอาหารเสริมอะฟลาทอกซิน บี 1 1 ไมโครกรัม/1 มก. ร่วมกับสารดูดซับชนิดต่างๆ ที่ระดับร้อยละ 0.5 (T6, T7, T8) ตามลำดับ ผลการศึกษาพบว่า ปริมาณแก๊สที่ผลิตจากส่วนที่ละลายยาก และปริมาณผลผลิตแก๊สที่ผลิตได้ทั้งหมดในอาหารที่มีของอะฟลาทอกซิน บี 1 ต่ำกว่าในอาหารกลุ่มควบคุม ($P<0.05$) นอกจากนี้ ยังพบว่ามอนต์โมริลโลไนต์ และยีสต์ *S.cerevisiae* มีประสิทธิภาพในการลดผลกระทบของของอะฟลาทอกซิน บี 1 ต่อการผลิตแก๊ส ในทางตรงข้ามอาหารที่เติมอะฟลาทอกซิน บี 1 และอาหารเสริมสารดูดซับสารพิษจากเชื้อราไม่มีผลกระทบต่อการย่อยได้ของวัตถุแห้งและการย่อยได้ของอินทรียวัตถุในหลอดทดลองปริมาณกรดไขมันที่ระเหยง่าย และแอมโมเนีย - ไนโตรเจน ($P>0.05$) สรุปได้ว่า การศึกษาครั้งนี้พบอะฟลาทอกซิน บี 1มีผลต่อกระบวนการหมักในหลอดทดลอง และการเสริมมอนต์โมริลโลไนต์ และยีสต์ *S.cerevisiae* ที่ระดับร้อยละ 0.5 เป็นสารดูดซับสารพิษจากเชื้อราพบว่ามีประสิทธิภาพสามารถลดผลไม่พึงประสงค์ของอะฟลาทอกซิน บี 1 ได้

คำสำคัญ: อะฟลาทอกซิน บี 1 สารดูดซับสารพิษจากเชื้อรา และ การหมักในกระเพาะรูเมน

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Introduction

Aflatoxins (AFs) are secondary metabolites commonly produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B1 (AFB1) is the most toxic and abundant aflatoxins (Jaynes and Zartman, 2011) contaminated in food or feeds in the field or during storage. AFB1 causes several toxicity as hepatotoxin, immunotoxin, carcinogenic and teratogenic to humans and animals (Jouany *et al*., 2009; Mojtahedi *et al*., 2013). Especially, AFB1 involved severe economic losses and health problems in dairy production because AFB1 is only partially degraded by rumen microorganisms. The remainder is then biologically converted to Aflatoxin M1 (AFM1) in the liver and eventually secreted in the dairy milk (Meucci *et al*., 2011). The carry over rate of AFB1 in feed to AFM1 in milk were 0.01–0.02 (Jiang *et al*., 2014). Moreover, previous studies reported that AFB1 decreased the gas production by affecting the rate and cumulative gas production (Jiang *et al*., 2012; Mojtahedi *et al*. (2013). Aaflatoxinalso decreased cellulolysis in rumen (Edrington *et al*., 1994).

As a result, various methods, including physical, chemical and biological method have been recently introduced to reduce or eliminate aflatoxin contaminated in feeds (Nemati *et al*., 2015). Moreover, the mycotoxin adsorbents become the most commonly used to decontaminate mycotoxin in animal feed including clays, activated carbons and yeast. These products are capable of attaching mycotoxins (Mojtahedi *et al*., 2013). Therefore, the objective of the study was to evaluate the effect of mycotoxin adsorbents for AFB1 decontamination and rumen fermentation efficiency by using *in vitro* gas production technique.

Materials and Methods

1. AFB1 Preparation

Commercially available AFB1 (Trilogy, Catalog number: TSL-401, USA) was prepared as stock standard solutions at 10 µg/ml using sterile deionized water.

2. Experimental design and treatments

The experimental design was conducted by using Completely Randomized Design (CRD). The basal diet containing 40% of rice straw and 60% of concentrate was allocated in eight treatments consisting T1=the control group, T2= the diet plus 1 µg/mg of aflatoxin B1 (AFB1), T3, T4, T5 = the diets supplement with 0.5% of each adsorbent (activated charcoal, montmorillonite, *S.cerevisiae*) and T6, T7, T8 = the diets plus 1 µg/mg of AFB1 together with 0.5% of each mycotoxin adsorbent, respectively. The dried basal diet was then milled, passed through a 0.2 mm sieve, and preceded to further step.

3. Rumen inoculums

Rumen fluid was collected from three healthy dairy cows, which were placed on a routine basis for at least 1 week and fed by the same basal diet for 5 days before sampling. *In vitro* fermentation was used in this study according to the technique described by Makkar *et al.* (1995). The rumen fluid (660 ml) was added to warm (39°C) and reduced medium consisting of 1,095 ml distilled water, 730 ml rumen buffer solution (417 mM $NaHCO_3$ and 51 mM NH_4HCO_3), 365 ml macromineral solution (46 mM KH_2PO_4, 40 mM Na_2HPO_4, 38 mMNaCl and 2 mM $MgSO_4 \cdot 7H_2O$), 0.23 ml micromineral solution (505 mM $MnCl_2 \cdot 4H_2O$, 898 mM $CaCl_2 \cdot 2H_2O$, 42 mM $CoCl_2 \cdot$

$6H_2O$ and 341 mM $FeCl_2 \cdot 6H_2O$), 1 ml of 4mM resazurin and 60 ml freshly prepared reduction solution (145 mM $Na_2S \cdot 9H_2O$ and 3.7 ml 1 M-NaOH). The mixture was kept stirring and continuously filled with $CO_2$to ensure anaerobic condition at 39°C on a hot plate. Approximately 30 ml of the rumen-fluid medium was transferred into serum bottles incubated with feed samples (200 mg) at 39 °C for 72 h.

4. Sample collection and analysis

Dietary treatments were analyzed for dry matter (DM), ash, crude protein (CP) and ether extract (EE) using the procedure of AOAC (1997), while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also determined as described by Van Soest *et al*. (1991).

5. Gas production

During the incubation, the gas production kinetics were recorded at 1, 2, 4, 6, 8, 12, 24, 48 and 72 h. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follow:

$$Y = a + b (1 - e^{(ct)})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production, y = gas production at time 't'. At 24- and 48-h post inoculation, a set of sample was determined *in vitro* true digestibility according to Van Soest and Robertson (1985).

6. Ammonia nitrogen and Volatile fatty acids analysis

Ruminal inoculum fluid was collected at 24 and 48 h after inoculation, about 30 ml of rumen inoculums mixed with 1M H_2SO_4 was centrifuged at 15,000×g for 15 min. Samples were divided into two portions for ammonia nitrogen (NH_3-N) analysis by using the micro-Kjeldahl methods (AOAC, 1990) and samples were then filtered through 0.22-μm nylon membrane (FILtrex, Singapore) for volatile fatty acids (VFAs) analysis by high performance liquid chromatography (HPLC) (model RF-10AXmugiL; Shimadze, Japan) according to Mathew *et al*. (1997).

7. Statistical analysis

All data were analyzed as a Completely Randomize Design using the SPSS version 19. The significant differences were compared by Post hoc test.

Results and Discussion

1. The chemical compositions

The chemical compositions of concentrate and rice straw in this study are shown in Table 1. The crude protein content in rice straw is 2.5%, 2.1 EE % and 83.0% OM, in accordance with previous report (Kang and Wanapat, 2013). Moreover, forage fiber analysis for rice straw in this experiment found that the rice straw contained 81.4% NDF, 50.8 % ADF and 5.9 ADL. The concentrate was formulated to contain 22.1% CP and 3.9% EE.

3. Gas production kinetic

Estimated parameters of gas production are presented in Table 2 and cumulative gas production profiles are shown in Fig. 1. In this

study, there were no treatment effect (P>0.05) on the fermentation of the soluble fraction (a) and effective gas production potential (EP). However, gas production from the insoluble fraction (b), gas production rate constant for the insoluble fraction and the potential extent of gas production were significantly different among treatments (P<0.05), of which the AFB1-containing diet was lower than the control group. These depressions in the gas production suggest that microbial populations are altered by AFB1 was add. This result was similar to previous studies (Jiang *et al*., 2012; Mojtahedi *et al*., 2013) where the asymptotic gas production numerically deceased by increasing AFB1 dosage.

The gas production from the insoluble fraction in the diet+AFB1 was lower than in the diet + AFB1 + montmorillonite and diet + AFB1 + *S. cerevisiae.* In addition, the gas production rate was the lowest in treatment plus AFB1. However, montmorillonite and *S. cerevisiae* were effectively decreased the AFB1 effect on gas production. This phenomenon could be explained through the detoxifying capability of *S. cerevisiae* cell wall and montmorillonite. The adsorption capacity largely depended on both yeast composition and mycotoxin as well. Joannis-Cassan *et al*. (2011) reported that the yeast cell wall from baker's yeast could absorb up to 29% of AFB1. Similarly, Desheng *et al*. (2005) also noted that the montmorillonite absorbed more than 80% of the available AFB1 from aqueous solution at pH 2 and 90% at pH 8, correspondingly previous report, where montmorillonite could absorb up to 93% of AFB1 (Dakovi *et al*., 2008).

4. Degradability and rumen metabolites

The *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) were not significantly affected by AFB1 and mycotoxin adsorbents (P>0.05) (Table 3). Likewise, Pettersson and Kiessling (1976) and Jiang *et al*. (2012) demonstrated that addition of AFB1 did not alter IVDMD. However, the result was opposed with Mojtahedi *et al*. (2013) who reported that IVDMD decreased significantly with AFB1 inclusion. It could be explained that AFB1 could attributed to compromise ruminal fermentation by reducing fiber digestion and VFA production.

Table 1 The chemical composition of rice straw and concentrate

Items	Rice straw	Concentrate
DM, %	94.3	91.9
Chemical composition	-------% of DM basis------	
OM	83.0	83.2
Ash	12.5	7.8
CP	2.5	22.1
EE	2.1	3.9
NDF	81.4	21.1
ADF	50.8	11.7
ADL	5.9	3.6

DM = Dry matter, OM = Organic matter, CP = Crude protein, EE = Ether extract

NDF = Neutral- detergent fiber, ADF = Acid- detergent fiber, ADL = Acid- detergent lignin

Table 2 Effects of AFB1 and mycotoxin adsorbents on *in vitro* gas production of dietary treatments

Items	Treatments								SEM	P-value
	T1	T2	T3	T4	T5	T6	T7	T8		
Gas production characteristics										
a	1.21	1.53	-0.64	-0.25	-0.48	-2.16	-1.55	-0.97	0.36	0.13
b	50.7^{bcde}	38.7^{a}	53.7^{cde}	44.2^{ab}	58.6^{e}	50.9^{abc}	56.2^{de}	49.5^{bcd}	1.54	<0.01
c	0.07^{ab}	0.18^{c}	0.08^{ab}	0.13^{c}	0.07^{ab}	0.12^{bc}	0.06^{a}	0.11^{abc}	0.01	0.02
d	51.9^{bc}	40.2^{a}	53.0^{bc}	44.0^{ab}	58.1^{c}	48.7^{abc}	54.6^{bc}	48.5^{abc}	1.52	0.04
Effective gas production potential (EP)										
	31.9	30.4	31.5	31.3	33.2	33.2	30.1	32.2	0.53	0.82

^{a,b,c,d,e}Means in the same row with different superscript differ (P<0.05)

a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction, d = the potential extent of gas production, EP = a + [bc/(k + c)] where k = 0.05 (Ørskov and McDonald, 1979).

T1=Basal diet (the control), T2= Basal diet plus AFB1 at 1 µg/mg, T3= Basal diet supplement with 0.5% activated charcoal,
T4= Basal diet supplement with 0.5% montmorillonite, T5= Basal diet supplement with 0.5% *S.cerevisiae*,
T6= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% activated charcoal,
T7= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% montmorillonite,
T8= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% *S. cerevisiae*

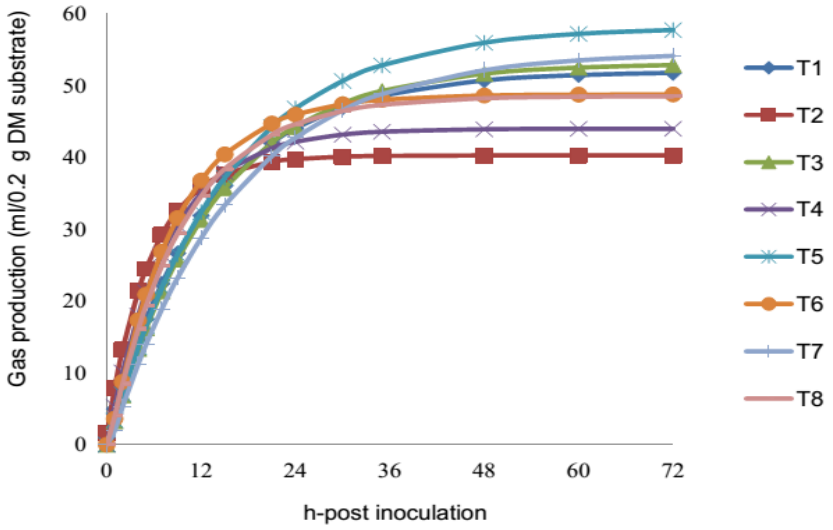

Fig. 1 Effect of AFB1 and mycotoxin adsorbents on gas production at different times of incubation in dairy cow's rumen fluid

T1=Basal diet (the control), T2= Basal diet plus AFB1 at 1 µg/mg, T3= Basal diet supplement with 0.5% activated charcoal,
T4= Basal diet supplement with 0.5% montmorillonite, T5= Basal diet supplement with 0.5% *S.cerevisiae*,
T6= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% activated charcoal, T7= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% montmorillonite, T8= Basal diet plus AFB1 at 1 µg/mg supplement with 0.5% *S. cerevisiae*

The results of VFAs (Acetic acid, Propionic acid, and Butyric acid) and NH_3-N are shown in Table 4. In this study, there were no effects of treatments on the VFAs and NH_3-N ($P>0.05$). However, some studies reported that the addition of AFB1 *in vitro* rumen fermentation significantly decreased NH_3-N concentrations (Mojtahedi *et al*. 2013; Jiang *et al*., 2012). AFB1 maybe was affected ammonia. Protein synthesis and increased the microbial requirement for nutrients (Edrington *et al*., 1994) when the digestion of protein was inhibited by AFB1, resulting in the decreased ammonia concentration.

Table 3 Effects of AFB1 and mycotoxin adsorbents on *in vitro* digestibility

Items	Treatments								SEM	P-value
	T1	T2	T3	T4	T5	T6	T7	T8		
In vitro dry matter digestibility (IVDMD), %										
24 h	48.9	49.5	49.5	47.5	50.7	48.9	53.2	52.2	0.64	0.42
48 h	58.9	59.2	58.0	62.1	56.2	61.8	57.7	62.2	0.38	0.55
In vitro organic matter digestibility (IVOMD), %										
24 h	54.8	54.2	55.3	53.8	56.5	56.1	58.0	56.8	0.52	0.58
48 h	65.4	65.2	63.7	66.9	63.2	66.1	64.1	66.1	0.63	0.89

T1=Basal diet (the control), T2= Basal diet plus AFB1 at 1 µg/mg, T3= Basal diet supplement with 0.5% activated charcoal, T4= Basal diet supplement with 0.5% montmorillonite, T5= Basal diet supplement with 0.5% *S.cerevisiae*, T6= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% activated charcoal, T7= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% montmorillonite, T8= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% *S. cerevisiae*

Table 4 Effects of AFB1 and mycotoxin adsorbents on volatile fatty acids and ammonia nitrogen

Items	Treatments								SEM	P-value
	T1	T2	T3	T4	T5	T6	T7	T8		
Total VFAs, mM										
24 h	112.7	108.1	109.1	125.9	111.5	91.3	122.3	126.3	3.28	0.07
48 h	133.5	122.6	127.8	137.2	120.2	128.1	131.5	127.8	2.40	0.80
Acetic acid (C_2), %										
24 h	58.9	55.1	56.1	58.1	56.5	53.0	58.0	58.2	0.67	0.41
48 h	60.5	58.1	59.8	59.8	58.9	59.2	59.4	59.3	0.37	0.92
Propionic acid (C_3), %										
24 h	25.7	32.4	30.5	30.0	29.5	33.1	27.8	27.7	0.79	0.27
48 h	27.1	27.9	27.9	28.2	28.9	28.0	27.2	27.2	0.28	0.84
Butyric acid (C_4), %										
24 h	15.5	12.5	13.5	13.0	14.0	14.0	14.3	14.1	0.27	0.17
48 h	12.4	14.1	12.2	12.0	12.2	12.8	13.3	13.6	0.22	0.10
C_2:C_3 ratio										
24 h	2.37	1.72	1.86	2.01	1.91	1.60	2.09	2.10	0.08	0.38
48 h	2.23	2.08	2.15	2.12	2.05	2.12	2.19	2.18	0.03	0.94
Ammonia nitrogen (NH_3-N), mg%										
24 h	17.6	17.0	18.2	15.2	15.2	18.2	17.0	18.0	0.29	0.39
48 h	18.9	29.1	18.8	18.5	19.5	20.1	21.3	21.6	0.36	0.20

T1=Basal diet (the control), T2= Basal diet plus AFB1 at 1 µg/mg, T3= Basal diet supplement with 0.5% activated charcoal, T4= Basal diet supplement with 0.5% montmorillonite, T5= Basal diet supplement with 0.5% *S.cerevisiae*, T6= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% activated charcoal, T7= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% montmorillonite, T8= Basal diet plus AFB1 at1 µg/mg supplement with 0.5% *S. cerevisiae*

Conclusion

In conclusion, our study found that AFB1 at 1 µg/mg affected *in vitro* fermentation characteristics by reducing the gas production from the insoluble fraction and potential gas production. However, the gas production from the immediately soluble fraction, EP, NH_3-N and VFAs concentrations did not different among treatments. In addition, montmorillonite and *S. cerevisiae* effectively reduced the effects of AFB1 on gas production.

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