Effect of mycotoxins contaminated corn on growth nutrient digestibility and *in vitro* rumen fermentation in goats

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Aflatoxins (AFs) are the toxic secondary metabolites produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which grow on numerous feedstuffs (mainly cereals) when environmental conditions are favorable. The major forms of AFs in feedstuffs include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). The order of toxicity is $AFB_1 > AFG_1 > AFB_2 > AFG_2$. AFB_1 is more common than the other AFs (Fandohan *et al*. 2005).

Ruminants seem to be more resistant to aflatoxicosis than monogastrics, but symptoms of aflatoxicosis were reported in bovine (Bodine and Mertens 1983, Cook *et al*. 1986, Diekman and Green 1992, Auerbach *et al*. 1998, Upadhaya *et al*. 2010). However, little information about the effects of AFB_1 on nutrient digestion and rumen fermentation is available (Jouany 2001). In addition, these studies were conducted mainly with purified toxins, but rarely with the naturally contaminated feed. In actual production, attention should also be paid to the modification of chemical and physical properties of feedstuffs caused by fungal invasion (Jouany *et al*. 2006, Seeling *et al*. 2006). Therefore, study was conducted to investigate the effects of corn naturally contaminated with aflatoxin B_1 on nutrient digestion and rumen fermentation of goats.

AFB_1-contaminated corns were collected from a feed factory in Sichuan by using a fluorescence detector. The corns naturally mildewed for 40 days at 27° to 30°C and 0.99 water activity. The types and concentrations of mycotoxins in the corns were detected by high performance liquid chromatography (HPLC).

The *in vivo* experiment was conducted at China Sichuan Lezhi Black Goats Sci-Tech Park from August to October 2012. Growing male China Lezhi black goats (12) with an average weight of 16.39 to 16.45 kg were equally divided to a trial group and a control group. Each group was kept in 6 folds (1 goat in each fold). All goats were fed with 40%

concentrated feed (60% corn) in the morning and 60% roughage (corn stover silage + rapeseed shell) in the afternoon and evening. The formula and chemical composition of the concentrate feed are shown in Table 1. The control group was fed with control corns free of mycotoxins; the trial group was fed with AFB_1-contaminated corns for 28 days.

The control corns and mycotoxin contaminated corns were fermented *in vitro* with goat rumen fluid for 3, 6, 12, 24 and 48 h, separately. Each sampling period was conducted in sextuplicate. The rumen fluids were collected from 4 China Lezhi black goats (32±5.3 kg), which were fed with 42.5% concentrate feed, 23.5% silage, 21% grass and 13% alfalfa hay. The rumen fluids were collected by using a rubber stomach tubet 0 hr post feeding and squeezed through eight layers of cheesecloth into an Erlenmeyer flask. The rumen fluids were gassed with oxygen-free CO_2 for 15 min and diluted with the McDougall buffer (McDougall 1948) at the ratio of 1:1. The corn samples were fermented *in vitro* with the rumen fluids in calibrated glass syringes following the procedures of Menke *et al.* (1979). The corn samples were milled to pass a 1 mm mesh and weighed 400 mg of DM into the 100 ml calibrated glass syringes. The syringes were pre-warmed at 39°C before injection.

Table 1. Formula and chemical composition of concentrate feed

Item	Diet	
	Control diet	AFB_1-contaminated diet
Ingredient	% as fed	
Corn	60.00	0
AFB1-contaminated corn	0	60.00
Commercial weaning feed	3.00	3.00
Silkworm faeces	23.53	23.53
Fermentation feed	7.90	7.90
Distiller's grains	5.57	5.57
Nutrient composition	%DM	
Crude protein (CP)	14.89	15.25
Crude fiber (CF)	10.00	10.16
Acid detergent fiber (ADF)	22.14	22.77
Neutral detergent fiber (NDF)	32.72	33.49

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Rumen fluid-buffer mixture (30 ml) was injected into each syringe. The corn samples were fermented in a thermostatic incubator at 39 ± 0.5 °C for 3, 6, 12, 24 and 48 h, separately.

The 100 g corn and diet were collected twice each week and stored at –20 °C. All of the corn samples were mixed at the end of experiment to quantify the mycotoxin contents by high performance liquid chromatography.

Growing goats were weighed at the beginning of the experiment and on day 28, separately. Feed (concentrated feed, silage and rapeseed shell) intake was recorded each day. Thus average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were determined. Abnormal behavior and daily mortality were recorded, and dead goats were dissected. Three goats in each group were killed on day 28 and the ruminal fluids were collected for measurements of pH value, ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA).

The concentrated feed, silage, rapeseed shell and fecal samples were collected at day 26, 27, 28. The samples of the 3 days were mixed and stored at –20°C for determination of dry matter (DM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and 4N Acid-insoluble ash (AIA). Apparent digestion coefficients were calculated by using the formula given by Church and Pond (1982) with AIA as the internal indicator. In the *in vitro* experiment, the volume of gas was measured at 3, 6, 12, 24 and 48 h after initiation of incubation. Total gas values were corrected by blank incubation. The pH, NH_3-N and VFA of fermentation fluid were measured at 3, 6, 12, 24 and 48 h.

The mycotoxins in corns and diets were quantified via HPLC. Separation was achieved on an SB-C18 column (4.6 × 250 mm; 5 μm). The mobile phases were methanol and water (45:55, v/v) for aflatoxins (AFB_1, AFB_2 and AFG_2); acetonitrile and water (15:85, v/v) for deoxynivalenol (DON); acetonitrile and water (80:20, v/v) for T-2 toxin; acetonitrile, water and methanol (46:46:8, v/v/v) for zearalenone (ZEN). The flow rate was 1 ml/min, and the injection volume was 50 μl.

For the feed and fecal samples, DM and CP were determined with AOAC (1990) procedures; CF, NDF, and ADF were determined with the procedure of Goering and Van Soest (1970); 4N AIA was determined with the procedure of Van Keulen and Young (1977).

For both ruminal fluid (*in vivo*) and fermentation fluid (*in vitro*), the pH values were measured immediately with a pH meter, the NH_3-N levels were detected with indophenol colorimetry (Zongci and Ming 2010), and VFA (acetic acid, propionic acid, butyric acid) concentrations were determined by using HPLC with a C_{18} column.

All data were analyzed by SPSS 18.0. *In vivo* statistical analyses were performed by using T-test Duncan, and *in vitro* statistical analyses were performed by using GLM with repeated statement. The fixed effects in the model were AFB_1 level, incubation time, and the interaction between AFB_1 level and incubation time. Individual incubation bottle nested within treatment was a random effect.

The gas production, gas production rate, pH and NH_3-N at each incubation time point were analyzed by using T-test. All means were considered significantly different at P<0.05.

The concentrations of mycotoxins in control corn, mycotoxin naturally contaminated corn, control diet, and mycotoxin naturally contaminated diet are given in Table 2. The mycotoxins in the control diet were below the detection limit. The contaminated diet had 74.49 μg/kg AFB_1, 2.08 μg/kg AFB_2, 59.71 μg/kg DON and 36.51 μg/ kg ZEN. Except AFB_1, the other mycotoxins were below the legal limits established by the European Union (European Commission 2010).

During the experiment, no typical clinical illness attributable to aflatoxicosis was observed. One goat in the control group died due to the parasitism of brain spore worm. The AFB_1-caused alterations in the growing goats' performance are shown in Table 3. The AFB_1-contaminated corn showed no significant effects on the intake of concentrated feed, silage, or rapeseed shell of goats (P >0.05) during the whole experimental period. The average daily gain (ADG) of the trial group was 81.4g, which was lower than the control group (20.2g/d, P=0.086). The FCR of the control group and the trial group were calculated to

Table 2. The concentrations of mycotoxins in corns and diets (μg/kg)

	Control corn	Mycotoxin-contaminated corn	Control diet	Mycotoxin-contaminated diet
AFB_1	N. D	289.54	N. D	74.49
AFB_2	N. D	19.54	N. D	2.08
AFG_1	N. D	10.34	N. D	ND
DON	76.49	165.01	N. D	59.71
ZEN	42.9	102.14	N. D	36.51
T-2	N. D	23.47	N. D	ND

ND, No detect.

Table 3. The effects of AFB_1 on the performance of growing goats

	Control group	AFB_1-corn group	P value
Daily feed intake (g/day)	923.86±48.42	905.78±60.49	0.142
Intake of concentrated feed (g/day)	372.18±32.62	375.35±13.24	0.831
Intake of silage (g/day)	460.75±14.56	454.45±39.92	0.747
Intake of rapeseed shell (g/day)	81.28±2.59	75.97±8.37	0.208
Initial body weight (kg)	16.39±0.74	16.45±0.5	0.638
Final body weight (kg)	19.09±1.09	18.83±0.20	0.83
Average daily gain (ADG), (g/day)	101.69±17.26	81.4±13.42	0.086
Feed conversion ratio (FCR), (g/g)	9.08±1.56	10.54±1.72	0.106

be 9.08±1.56 and 10.54±1.72, respectively. AFB_1 improved the feed conversion ratio and decreased the feed conversion efficiency in growing goats (P >0.05).

The effects of AFB_1 on nutrient digestibility of growing goats are shown in Table 4. The CP digestibility of the growing goats fed with AFB_1-contaminated corn was significantly reduced by 7.05 % (P <0.05) compared to the control group. The digestibility of DM, CF, NDF or ADF had no significant change (P >0.05). Ruminants, however, are considered generally more resistant to the adverse effects of mycotoxins, because the rumen microbiota could bio-transform the mycotoxins. But some of the metabolites of mycotoxins are still toxic (Fink-Gremmels 2008). In this study, the aflatoxin had no significant effect on feed intake of growing goats, but the body weight gain and feed consumption were lower (P >0.05). Similar results were

found in ruminants like lamb (Fernaindez 2000), deer (Quist *et al.* 1997), steer (Richard *et al.* 1983) and dairy cow (Korosteleva *et al.* 2009).

Mycotoxin contamination may change the digestibility of individual feed components (Gremmels *et al.* 2008). Many experiments about mycotoxin were for the absorbents, but some experimental design had no normal feed treatment. For example, Kiyothong *et al.* (2012) reported that the pH, NH_3-N, VFA, amounts of rumen bacteria, and the digestibility of CP, NDF were significantly (P <0.05) higher in mycotoxin deactivator supplemented than in cows fed with contaminated mycotoxin, and some of those indexes were not within the normal physiological range. Therefore, if we could suppose that the mycotoxin had some negative influence on the metabolism of cows. In this study, the AFB_1 significantly reduced the CP

Table 4. The effects of AFB_1 on the nutrient digestion of growing goats

	DM (%)	CP (%)	CF (%)	ADF (%)	NDF (%)
Control group	61.52±2.30	60.74±2.41[*]	52.83±3.77	62.46±4.32	61.54±3.63
AFB_1-corn group	59.88±2.61	56.46±2.72	54.65±4.58	59.44±7.15	57.02±10.97
P value	0.419	0.035	0.531	0.515	0.456

*, P<0.05.

Table 5. The effects of AFB_1 on the rumen fermentation of growing goats

	pH	NH_3-N (mg/dl)	acetic acid (mmol/l)	propionic acid (mmol/l)	butanoic acid (mmol/l)
Control group	7.32±0.04	8.34±0.74[*]	44.52±1.70	10.93±0.42	5.76±0.69
AFB_1-corn group	7.22±0.04	6.21±0.03	43.04±3.32	10.05±0.48	5.51±0.99
p value	0.441	0.046	0.631	0.194	0.796

*, P<0.05.

Table 6. *In vitro* rumen fermentation parameters

	3h	6h	12h	24h	48h	SEM	P value [a]		
							Time	AFB_1	Time×AFB_1
Gas (ml)									
Control group	11.45[**]	47.14[**]	83.33[**]	90.67	97.67	3.738	<0.001	<0.001	0.015
AFB1-corn group	1.82	16.67	64.67	75.00	90.67				
T-test P value	<0.001	<0.001	0.005	0.06	0.588				
Gas rate (ml/h)									
Control group	3.82[**]	7.86[**]	6.94[**]	3.78	2.11	0.342	"ÿ0.001	<0.001	<0.001
AFB1-corn group	0.61	2.78	5.39	3.13	1.89				
T-test P value	<0.001	<0.001	0.005	0.06	0.588				
pH									
Control group	7.28	6.88	6.47	6.23	5.88[*]	0.178	"ÿ0.001	0.105	0.332
AFB1-corn group	7.15	6.87	6.44	6.16	5.60				
T-test P value	0.178	0.67	0.833	0.136	0.045				
NH3-N (mg/dl)									
Control group	17.52	10.88[**]	5.43[**]	4.30[**]	3.26	0.931	<0.001	<0.001	0.003
AFB1-corn group	15.56	15.06	13.37	8.73	5.83				
T-test P value	0.486	0.001	0.001	0.008	0.252				

*, P<0.05; **, P<0.01.[a], Time, fermentation time; AFB_1, AFB_1 contamination treatment; time×AFB_1, time×AFB_1 contamination treatment.

digestibility (P <0.05). Rao (2003) reported that AF administration (300 ppb) resulted in lower CP digestibility (P <0.01), and it was not improved even by supplementation with Sodium Bentonite or Activated Charcoal. The same result was reported in duck (Han *et al.* 2008) and laying hens (Kermanshahi *et al.* 2007). AFB_1 could inhibit the rumen microbial activity, such as *Streptococcus bovis* and *Bacillus megaterium,* and affect rumen microbial flora diversity, such as four different strains of *B. fibrisolvens* (Westlake *et al.* 1987, Kiessling *et al.* 1984, Mertens *et al.* 1979). Those studies showed that the AFB_1 inhibited rumen microbial fermentation. Cook *et al.* (1986) reported that aflatoxin could change the rumen motility in steers, like the amplitude (strength) and frequency of rumen contractions decreased. Therefore, AFB_1 may affect nutrient digestibility in ruminants by those ways.

The activities of carboxymethyl cellulase and microcrystalline cellulose in dairy cow rumen fluid were inhibited by AFB_1 *in vitro* (Jiang *et al*. 2010). Dvorak (1977) reported that aflatoxin decreased cellulose digestion in dairy cows. In this study, the NDF and ADF digestibility decreased too, but not significantly (P >0.05).

The rumen fermentation of growing goats is showed in Table 5. The NH_3-N content of the trial group (6.21±0.03 mg/dl) was significantly lower than the control group (8.34±0.74 mg/dl) (P <0.05). The pH and fractions of VFA were similar in control and treatment group in the trial group were lower than the control group, but not significant (P >0.05).

The *in vitro* effects of AFB_1 on gas production, gas production rate, rumen fluid pH value and NH_3-N are shown in Table 6. The gas production and gas production rate were significantly influenced by the incubation time, AFB_1 and the interaction between incubation time and AFB_1 (P <0.05). The gas production increased with the prolongation of incubation time (P <0.01). During fermentation period from 0 to 3 h, the cumulative gas production of the AFB_1-corn group (1.82 ml) was significantly lower than that of the control group (11.45 ml) (P <0.01). However, the total gas production showed no significant difference between the control group (97.67 ml) and AFB_1-corn group (90.67 ml) (P=0.588).The gas production rate of the control group was always higher than the AFB_1-corn group in the incubation period from 0 to 48 h. The highest gas production rate appeared at 6 h (7.86 ml/h) in the control group and later at 12 h (5.39 ml/h) in the AFB_1-corn group.

AFB_1 had no significant effect on pH (P=0.105). The pH value became increasingly lower with extension of incubation time (P <0.001). At 48 h, the pH values of the control group and AFB_1-corn group were 5.88 and 5.6, respectively. Because the incubation metabolic products were accumulated, the pH at 48 h was not within the physiological rumen pH range (6 to 7).

The NH_3-N levels in the two groups continued to decrease in total fermentation (P <0.001), and the decline in the AFB_1-corn group was slower than in the control group. The two groups showed no significant differences at 3 h or 48 h. But at 6 h, 12 h and 24 h, the NH_3-N levels in the AFB_1-corn group were significantly higher than in the control group (P <0.001). And there was a significant interaction between incubation time and AFB_1 content (P =0.003).

NH_3-N is the decomposition product of rumen nitrogenous substances. Under adequate supply of soluble carbohydrate and energy, NH_3-N could be synthesized for bacterial protein by rumen microorganisms. In this *in vitro* experiment, the NH_3-N content of AFB_1-corn group was lower than the control group (P >0.05) at 0 h to 3 h. The result was similar to the *in vivo* experiment and consistent with the lower degradation rate of crude protein of the AFB1-corn group in the digestion test too. This was because the rumen microorganism was inhibited by AFB_1, so the nitrogenous substance in corn could not be effectively degraded to NH_3-N. But the *in vitro* trial was a closed system, the large amount of NH_3-N produced by the rumen microorganism fermentation at early stage cannot be absorbed by the rumen wall. And the ability of microbial protein synthesis was weakened by AFB_1. So the NH_3-N content of the AFB_1-corn group was higher than the control group *(*P <0.05) in the late stage of *in vitro* fermentation.

The pH value and VFA had no significant changes (P >0.05). Edrington *et al.* (1994) reported that after goats were fed with 2.5 mg of AF kg diet for 35 days, the rumen pH and VFA had no significant changes. Helferich *et al.* (1986) got similar results on steer. But Bodine and Mertens (1983), Jiang (2010) presumed that rumen microorganisms were very sensitive to aflatoxin, the VFA production and proportion were changed by aflatoxin *in vitro* test. The different results may be related to the dose, animal species and differences between naturally contaminated mycotoxin and pure mycotoxin.

Gas production and gas production rate are intuitive indicators of rumen fermentation. In this *in vitro* experiment, the gas production and gas production rate in the AFB_1-corn group were both significantly lower than in the control group (P <0.01). It showed that the AFB_1 inhibited rumen microbial fermentation. Westlake *et al.* (1989) reported that with the addition of 1~10 μg/ml aflatoxin M, the rumen microbial digestion decreased by 50 to 67%. The reason may be that the rumen microbes in fermentation fluid changed. In addition, we found that the incubation time and AFB_1 content had significant effects on the rumen fermentation of goats. As early as 0 h to 3 h, the gas production and gas production rate of the AFB_1-corn group extremely significantly decreased, which indicated that AFB_1 may affect the rumen fermentation in the initial administration.

Corns naturally contaminated with harmful mycotoxins had no significant effect on the feed intake of growing goats, but decreased the nutrient digestibility and adversely affected the *in vitro* rumen fermentation. These lead to the reduction of ADG and feed consumption. Although ruminants have certain tolerance to mycotoxin, the rumen is negatively affected by mycotoxins when the rumen

degrades the mycotoxin. Accordingly, mycotoxin contamination on ruminant feed cannot be ignored.

SUMMARY

Two trials (*in vivo* and *in vitro*) were conducted to evaluate corn naturally contaminated with mycotoxins, majority being aflatoxin B_1 (AFB_1) on the performance, nutrient digestion and rumen fermentation in growing goats. China Lezhi black goats (12), weighing 16.39 to 16.45 kg, were fed with the diet of 40% concentrate (the mycotoxin naturally contaminated diet containing 74.49 μg/kg AFB_1, 2.08 μg/kg AFB_2, 59.71 μg/kg DON and 36.51 μg/kg ZEN) for 28 days. The results showed that the contaminated corn had no significant effect on feed intake but decreased the average daily gain (ADG) and feed conversion ratio (FCR) in growing goats. Digestibility of crude protein (CP) in the trial group was significantly lower than the control group and the digestibilities of acid detergent fibre (ADF) and neutral detergent fibre (NDF) decreased too, but not significantly. Neither volatile fatty acid (VFA) nor pH was significantly different between the 2 groups. The ammonia nitrogen (NH_3-N) in the trial group was lower in both *in vivo* trial and *in vitro* trial (0 h to 3 h). In *in vitro* experiment, ruminal fluids were collected from 4 China Lezhi goats and incubated at 39°C for 48 h with control corn or AFB_1 contaminated corn. The total gas production and gas production rate in the trial group were significantly lower than the control group. These reductions showed the negative effects of the naturally contaminated AFB_1 corn on nutrient digestibility and rumen function in growing goats.

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BOOK REVIEW

Parasitic Zoonoses in the New Millennium. 2015. M.B. Chhabra and K.M.L. Pathak. Today & Tomorrow's Printers and Publishers 4436/7, Ansari Road, Daryaganj, New Delhi 110012. 15.5 cm×23.5 cm. pp i – xii + 1-269. ISBN: 81-7019-519-1 (India), 1-55528-575-6 (USA).

Parasitic zoonoses or diseases caused by parasites transmissible between men and animals, is one of the major problems faced by humanity worldwide. In recent times, it has been exacerbated by several environmental and human factors, thereby enhancing the risk perceptions. Global agencies like WHO, and FAO, through a series of Expert Committee Reports, have brought increased attention and heightened concern on the impact of these diseases on the health and economy of vast populations. Parasitic Zoonoses is a highly relevant and rapidly-evolving sub-discipline of parasitology involving both medical and veterinary interesting.

The contents are arranged in six sections: first is Introduction which includes different classification infectious diseases, epidemiology and socio-economic impact of zoonoses. The other five sections deal with 52 diseases under major groups of etiological agents, namely Protozoa (14), Trematoda (10), Cestoda (8), Nematoda (14) and Arthropod zoonoses (6). Each disease has been described under appropriate sub-headings, namely synonyms, etiology, history (where available), life-cycle, epidemiology, clinical syndrome in man and animals, diagnosis and control who may wish to do additional reading. The Illustrations and Data are given to explain the facts in 10 Tables. In view of the emerging significance of wildlife as additional reservoirs of zoonoses and Appendix listing the various zoonotic parasites recorded from different willdlife species in India, has been added.

Coming at a time palpable paucity of comprehensive texts on this subject, particularly in India, the present authors deserve kudos for their effort to fill the void. This book scores over its contemporaries in certain special features. In keeping with its title, most of the literature cited pertains to the post-2000 period, material has been drawn from both medical and veterinary sources and despite its worldwide outreach, the Indian perspective has given special emphasis. It is heartening to note that the often-neglected area of arthropod zoonoses has been given adequate attention. Emerging (and re-emerging) zoonoses like cryptosporidiosis, toxoplasmosis, cysticercosis, hydatidosis and myiases like have been allotted greater space in proportion to their growing importance. The certain disease entities like fascioliases, larva migrans and zoonotic gilariases, have made progressive in roads in parts of India. The language is simple, lucid and concise. The overall get-up and production of the book is excellent. High quality printing and aesthetically-pleasing cover design, add to the value of the book. The book will be eminently suitable reference manual for post-graduate, as well as advanced undergraduate students of both veterinary and human medicine, parasitological and public health in particular, and those of biomedical sciences, in general.

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