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Original article

Growth response and nutrient digestibility of West African Dwarf goats fed micro doses of dietary aflatoxin

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ABSTRACT

An experiment was conducted with twenty West African Dwarf (WAD) goats (4-5 months old) averaging 8.40±0.2kg to assess performance and nutrient digestibility of WAD goats exposed to varied levels of dietary aflatoxin of 0ppb, 50ppb, 100ppb and 150ppb in concentrate diets 1, 2, 3 and 4 respectively for a period of 12 weeks. Aflatoxin contaminated maize was used with uncontaminated maize to vary the level of aflatoxin concentration in the concentrate diets. Feed intake, body weight and weight gain were monitored throughout the experimental period and the nutrient digestibility in the experimental animals was determined. Results showed that the average weight gain (g/goat) at the end of the trial declined with increasing aflatoxin level in the diets by 50.97%, 64.70% and 76.48% in animals fed diets 2, 3 and 4 respectively compared to the mean weight gain of the control (36.4g). The feed consumption of goats fed control diet was different from diet 4, but both were higher than those fed diets 2 and 3. Feed intake of goats fed diet 3 was higher than those on diet 2. Forty percent mortality was recorded in each of treatments 2, 3 and 4. Nutrient digestibility was influenced by dietary aflatoxin, but no consistent trend was observed with increase in aflatoxin concentration. These results suggest dietary aflatoxin up to 50ppb impaired performance of WAD goats. It also impaired nutrient digestibility, utilisation and depressed growth most especially when fed at 100 ppb and 150ppb to WAD goats.

1. Introduction

Aflatoxins are one of the most potent toxic substances that occur naturally. These are a group of closely related mycotoxins produced by fungi *Aspergillus flavus* and *A. Parasiticus*. *Aspergillus flavus* and *A. parasiticus* colonize a wide variety of food commodities including maize, oil seeds, spices, groundnuts, tree nuts, milk, and dried fruit (Strosnider *et al*., 2006). Aflatoxicosis is poisoning that result from ingestion of aflatoxins in contaminated food or feed. Of all foodstuffs, maize and peanuts (Wu and Khlangwiset, 2010) are the main sources of mammalian exposure to aflatoxin because they are consumed worldwide and unfortunately they are the most susceptible crops to aflatoxin contamination. Aflatoxin poisoning is reported from all parts of the world in almost all domestic and non domestic animals (ruminant and monogastrics). When aflatoxin is consumed, it can exert toxicity in several ways. It may alter intestinal integrity (Gong *et al*., 2008). Chronic aflatoxin exposure in animals can cause reduced feed conversion efficiency, increased mortality rates, reduced weight gain, induced anemia, and jaundice.

Ruminants are exposed to mycotoxin in growing pasture (fungal contamination of grasses), Silage, Hay and straw (including bedding), and concentrate feed formulated with contaminated grains. Mycotoxins affect ruminants by reducing feed consumption, reducing nutrient utilization, altering rumen fermentation, suppressing immunity, altering reproduction, irritating tissues, and causing cellular death (Agag, 2004). A single large dose of a mycotoxin can cause an acute toxicity in ruminant, but it is more likely that the effects are chronic, caused by low-level consumption over time. Some evidence on aflatoxicosis shows an effect on rumen microflora, this is characterized by a decrease in cellulolysis, Volatile Fatty Acid production, and ammonium formation resulting from reduced bacteria population.

Concentrate supplementation in ruminant diet is essential to improve rumen activities and the performance of the animal. However, incidence of aflatoxins contamination in grains used in formulating concentrate is increasing daily. Therefore, aflatoxin contamination can jeopardise the aim of fortifying ruminant diet with concentrate for optimal performance. Animal response to aflatoxin toxicity differs, as some animals are more resistant than others. Susceptibility of small ruminants indigenous to the tropics to aflatoxicosis is still a debate with little or no literature. In light of these, there is need for the evaluation of growth response of West African Dwarf goats to dietary aflatoxin exposure.

2. Materials and methods

2.1. Aflatoxin contaminated maize grains

Maize grains served as the aflatoxin carrier in the experimental diets for this study. The maize grains used for this experiment was obtained, autoclaved and inoculated with toxigenic *Aspergillus flavus* predominant in Nigeria. This culturing and inoculation was done at the Plant Pathology Unit, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The spores of the fungus producing aflatoxin (Aspergillus flavus) was prepare for growth on 5'2 medium 5/2 agar medium (5% V8 juice and 2% agar, pH 5.2). Growth of fungal spores was arrested after 7days and spore solution was prepared by washing off spores, into 0.1% Tween 20. Clean maize grains were soaked for 6 hours, after which were packed in autoclave nylon and autoclaved at 121^0c for 20 minutes. After autoclaving, grains were allowed to cool and inoculated with Aspergillus spore inoculums and packed in sealed nylon. The inoculated maize grains were transferred to screen house for incubation for `14 days. The maize kernels were washed off spores by adding 0.1% Tween 80. The washed grains were packed to another screen house for even heat distribution and drying. After the grains were well dried, samples were taken for aflatoxin analysis.

2.2. Determination of aflatoxin concentration

A homogenous grains for aflatoxin analysis was obtained by quarter sampling and the maize samples were ground and the samples from the four diets to a particular size less than 2mm with test portion sizes of 200g.

A 20g sub-sample from a bulk sample of 200g was ground and extracted with 100 ml of 70% methanol using a high-speed blender (Waring Commercial, Springfield, MO) for 3 min. The mixture was then passed through

Whatman paper No. 1, and the extract collected in a 250ml separating funnel and 100 ml of distilled water was added to ease separation. The solution was extracted twice with 25 ml methylene chloride. Following separation, the methylene chloride layer was filtered through 40 g of anhydrous sodium sulphate to remove residual water. The extract was collected in a polypropylene cup and evaporated to dryness in a fume hood. The residue was redissolved in 200µl of methylene chloride and either diluted or concentrated to allow accurate densitometry. Extracts and aflatoxin standards were separated on thin-layer chromatography (TLC) plates (silica gel 60, 250 µm) by development with diethyl ether-methanol-water (96:3:1), visualized under ultraviolet light, and scored visually for presence or absence of aflatoxin with a 2 mg limit of detection. Aflatoxins were quantified using scanning densitometer, CAMAG TLC Scanner 3 with win- CATS 1.4.2 software (Camag AG, Muttenz, Switzerland), as described previously by Suhagia et al. (2006).

2.3. Experimental materials and feeding trial

The Aflatoxin contaminated maize was substituted for non contaminated maize in various proportions to formulate four treatment diets containing approximately 0ppb, 50ppb, 100ppb and 150ppb in diets 1, 2, 3 and 4 respectively.

Twenty pubertal WAD goats of 4-5 months old weighing 8.40 ± 0.2kg were assigned randomly by weight, to the four diets in a 12-week feeding trial. Animals were fed concentrate as supplement to gliricidia sepium (40:60). Dietary treatments were offered to the respective animals twice daily. Feed supply was adequate and responsive to the goats weight changes, since feed consumption would be expected to change with body weight. The gross composition of the diets is shown in Table 1. All diets were isonitrogenous and isocaloric.

2.4. Digestibility trial

Animals were housed individually in metabolic cages which allows for separate urine and faecal collection. Diets and water were offered. Diets and faeces were analysed for proximate composition and urine nitrogen as described by AOAC (2000), and fibre fractions according to Van Soest et al. (1991).

2.5. Data analysis

All data obtained were subjected to statistical analysis using analysis of variance of statistical analysis software (SAS, 1999). Treatment means were compared using Duncan's multiple range test option of the same software.

3. Results

3.1. Growth performance of WAD goats

The performance data on the initial and final live weights, average weekly and daily weight gain, daily intake of concentrate, forage and total feed on dry matter basis and feed conversion ratio of the WAD goat are as shown in Table 2. The performance of WAD goats was influenced by the dietary treatments. The final live weights of the animals declined with increase in the aflatoxin levels in the diets. The daily weight gain of goats fed experimental diets decreased with increase in dietary aflatoxin levels, with the highest value (36.4g/goat) recorded in goats fed control diet and least weight (8.57g/goat) was recorded in goats on diet 4 (150ppb).

Daily concentrate dry matter intake of the goats fed control diet was not significantly different from goats fed diet 4 but was higher than goats fed diets 2 and 3. However, forage dry matter intake of goats fed dietary treatments was not different from one another. Daily concentrate intake and total dry matter intake of the goats fed control diet was not different from goats fed diet 4, but both were higher than goats fed diets 2 and 3, while daily concentrate intake and total dry matter intake of goats fed diet 3 were higher than goats fed diet 2. Feed conversion ratio of the animals increased with increase in aflatoxin level in the diets, with the highest value (15.5) recorded in animals fed diet 4 and least value (3.68) recorded in animals fed the control diet. Forty percent mortality was recorded in each of treatments 2, 3 and 4.

3.2. Nutrient digestibility of WAD goats

Nutrient digestibility (dry matter, crude protein, crude fibre, ether extract, ash, nitrogen free extract, and fibre fractions) of goats fed varied levels of dietary aflatoxin is as shown in Tables 3 and 4 respectively. Digestibility

of nutrients was influenced by dietary treatments except crude protein of concentrate-forage mixture and nitrogen free extract of concentrate, which were similar among the treatments. Digestibility values of dry matter, crude protein, ether extract, crude fibre and ash of concentrate-forage mixture in animals fed control diet were not different from goats on diet 4, but higher than that of goats on diet 2. Goats on diet 2 had higher values than those on diet 3.

Crude protein digestibility of concentrate-forage mixture had the highest value for goats fed control diet followed by animals on diet 4 and decline from goats fed diet 2 to diet 3. Crude protein digestibility of concentrate diet in animals fed control diet was not different from those on diet 4, but both were higher than goats fed diets 2 and 3. Fibre fraction digestibility of concentrate-forage mixture in animals on diet 2 was not influenced by dietary treatments. Animals on control diet and diets 2 and 4 had similar values for NDF digestibility and these values were higher than those recorded for animals fed diet 3. The ADF and ADL digestibility of concentrate and concentrate-forage mixture were higher for animals on diet1 than animals on diet 4 which had least value.

Table 1
Gross composition (%) of concentrate diet.

Ingredients	T1 (0ppb)	T2 (50ppb)	T3 (100ppb)	T4(150ppb)
Uncontaminated maize	55.0	51.7	48.3	45.0
Contaminated maize	0.00	3.33	6.66	9.99
Wheat offal	29.0	29.0	29.0	29.0
Brewer dried grain	15.0	15.0	15.0	15.0
Di-calcium phosphate	0.25	0.25	0.25	0.25
Grower premix	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Calculated nutrient value				
Crude protein (%)	12.9	12.9	12.9	12.9
DE (kcal/g)	3.57	3.57	3.57	3.57
NDF	25.4	25.4	25.4	25.4
ADF	9.01	9.01	9.01	9.01

DM: dry matter, DE: Digestible Energy; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre.

Table 2
Performance of WAD goats fed micro doses of dietary aflatoxins.

	Treatments				
Parameters	**1 (0ppb)**	**2 (50ppb)**	**3 (100ppb)**	**4 (150ppb)**	**±SEM**
Initial weight (kg)	8.40	8.40	8.40	8.40	
Final weight (kg)	11.5^{a}	9.90^{b}	9.48^{bc}	9.10^{c}	0.597
Concentrate DMI (g/day)	166^{a}	138^{c}	149^{b}	162^{a}	3.66
Forage DMI (g/day)	57.2	51.3	53.4	58.5	9.40
Total DMI (g/day)	223^{a}	189^{c}	203^{b}	221^{a}	4.50
Daily weight gain (g)	36.4^{a}	17.9^{b}	12.9^{bc}	8.57^{c}	5.78
FCR	3.68^{d}	6.36^{c}	9.47^{b}	15.5^{a}	1.54
Mortality (%)	0.00	40.0	40.0	40.0	10.5

abc: means in the same row with different superscripts are significantly ($P<0.05$) different.
SEM: Standard Error of Mean; DMI: Dry Matter Intake; FCR: Feed Conversion Ratio.

4. Discussion

Upon aflatoxin ingestion, it alters intestinal integrity (Gong *et al*., 2008), induces immune suppression, stunted growth and mortality in human and animal species (Wu, 2010). This study provides evidence of growth response of WAD goats to varied dietary aflatoxin levels. The mean daily concentrate dry matter intake of WAD goats fed control diet and 150ppb aflatoxin compared favourably. However, the daily forage dry matter intake of

goats fed 150ppb aflatoxin was apparently higher than goats fed control diet though both were statistically similar. The statistically identical dry matter intake among goats fed control diets and 150ppb aflatoxin may probably be attributed to physiological adjustment of the animals to the feed, which makes them consume the diets in order to satisfy their nutritional requirement irrespective of the toxin assault. This probably account for lower feed intake as toxin level reduced, resulting from less toxin assault compared to animals fed 150ppb aflatoxin. The feed consumption of animals fed 150ppb aflatoxin that was similar to those on the control diet, may be probably due to aflatoxin in the diet, which lowered the quality of the diets, promote the increase in the dry matter intake of animals fed 150ppb aflatoxin which compared favourably with the control animals in attempt to meet their nutrient requirement. This could be attributed to diet supplemented with multipurpose browse plant (Gliricidia sepium; highly nutritious forage for ruminant), who's intake was highest in animals fed 150ppb aflatoxin diet. The result obtained from this study was at variance to the reports of Edrington et al. (1994), who reported reduced feed intake in lambs fed concentrate (total mixed ration) diet contaminated with aflatoxin, probably as a result of no forage supplement was fed to the lambs.

Table 3
Nutrient digestibility of WAD goats fed varied levels of dietary aflatoxin (Concentrate and forage mixture).

		Treatments			
Parameters	**1 (control)**	**2 (50ppb)**	**3 (100ppb)**	**4 (150ppb)**	**±SEM**
Dry matter:	74.5^{a}	71.0^{b}	60.7^{c}	75.9^{a}	3.07
Crude protein	36.0	28.0	25.8	30.1	9.02
Crude fibre	60.1^{a}	49.7^{b}	30.9^{c}	56.7^{ab}	5.54
Ether extract	80.2^{a}	68.5^{b}	67.5^{b}	79.1^{a}	3.06
Ash	77.6a	72.6a	66.9^{b}	77.6^{a}	2.54
NFE	87.9^{b}	91.6^{a}	86.7^{b}	91.9^{a}	1.29
NDF	73.8^{a}	73.3^{a}	58.02^{b}	77.48^{a}	3.35
ADF	43.4^{a}	38.7^{ab}	32.3^{b}	16.0^{c}	7.33
ADL	64.7^{a}	59.8^{a}	39.2^{b}	36.5^{b}	16.6

abc: means in the same row with different superscripts are significantly (P<0.05) different.
SEM: Standard Error of Mean; NFE: Nitrogen Free Extract; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin.

The mean final liveweight and daily weight gain of goats fed dietary treatments declined with increase in the levels of aflatoxin concentration in the diets. The percentage reduction in weight gain of animals fed 50ppb, 100ppb and 150ppb increased to about 51.0%, 64.7% and 76.5% respectively relative to the mean daily weight gain of control animals (36.5g), after 12 weeks of dietary exposure. This result is in agreement with findings of Marina et al. (2002) who reported a dose related effect of aflatoxin upon body weight gain of piglets. The reduction in weight gain may be attributed to the influence of aflatoxin, since all the experimental diets were isonitrogenous and isocaloric. Diaz et al. (2008) reported biphasic nature of effects of aflatoxin on weight gain in broiler, in which improvement at low doses and reduction in weight gain at high doses. Yunus et al. (2011) reported that level and length of exposure determine the performance via percentage reduction in weight of broilers. They suggested that it is impracticable to generalise the dose response relationship with weight gain.

Feed conversion ratio of WAD goats increased with increase in the dietary aflatoxin levels. This implies that aflatoxin may likely inhibit nutrient absorption and utilisation. The rate of conversion of feed to gain in animals fed 50ppb, 100ppb and 150ppb was depressed to about 42.1%, 61.1% and 76.3% respectively, relative to the mean feed conversion ratio of control goats. This probably suggests the reason for the declined body weight gain observed among WAD goats with increase in dietary aflatoxin concentration, since all the animals fed control diet and 150ppb have identical dry matter intake. The results of this finding corroborates Cheeke and Shull (1985) who reported decreased feed efficiency and decreased growth rate as the most common effects of chronic aflatoxicosis in young livestock, and Richard et al (1983) reported decreased performance (rate of gain) in steers fed aflatoxin contaminated diets. Radostits et al. (2000) reported that, Levels of aflatoxin in excess of 100 µg/kg of feed are considered to be poisonous for cattle. Previous researchers reported that, Aflatoxins impair swine performance by reducing feed efficiency and rate of growth (Diekman *et al*., 1992), body weights of the intoxicated piglets were

lowered after 3 and 4 weeks of intoxication (Marin *et al*., 2002). Harvey et al. (1991) reported a dose of 2.6 mg of AFB1/ kg diet significantly reduced feed intake and body weight gain. Abdel-Fattah et al. (2010) reported average body weights, body weight gains and feed conversion ratio were affected during the exposure of sheep to aflatoxin. Richard et al. (1983) reported decreased rate of gain as one of the most sensitive indicator of aflatoxicosis. The ultimate cause of this effect may probably be multifactorial, involving not only nutritional interactions, but also the compounding influences of anorexia, deranged hepatic protein, lipid metabolism and disturbances in hormonal metabolism as reported by Raisbeck et al. (1991), which probably accounted for the percent mortality during the last 10 days of the feeding trial in animals supplemented with concentrate contaminated with aflatoxin in this study, has also reported by Wu (2010).

Nutrient digestibility of concentrate and whole feed of animals on aflatoxin diets was lower compared to the control diet, but no consistent pattern could be drawn. Nelson et al. (1982) reported that, Aflatoxin did not affect digestibility of some nutrient. Some studies indicate that the reduced nutrient utilisation observed in animals might be due to the effect of the toxin on systemic metabolism rather than digestive functionality (Verma *et al*., 2002; Verma *et al.,* 2007). Aflatoxin has been believed to result in malabsorption of macro nutrient and reduced activity of digestive enzyme (Surai and Dvorska., 2005; Devegowda and Murthy, 2005).

Table 4
Nutrient digestibility of WAD goats fed varied levels of dietary aflatoxin (Concentrate diet).

		Treatments			
Parameters	**1 (control)**	**2 (50ppb)**	**3 (100ppb)**	**4 (150ppb)**	**SEM**
Dry matter	83.3^{a}	76.3^{b}	70.6^{c}	76.9^{b}	3.31
Crude protein	53.0^{a}	34.3^{b}	13.1^{c}	41.3^{a}	9.88
Crude fibre	66.2^{a}	53.9^{b}	42.5^{c}	58.5^{ab}	6.31
Ether extract	60.4^{a}	42.6^{c}	38.4^{c}	50.6^{b}	7.18
Ash	86.3^{a}	78.6^{b}	77.9^{b}	80.0^{b}	2.89
NFE	95.7	94.3	93.6	93.3	0.88
NDF	83.8^{a}	77.3^{b}	70.6^{c}	78.3^{b}	3.28
ADF	34.0^{a}	23.8^{a}	7.05^{b}	9.21^{b}	11.7
ADL	72.9^{a}	68.8^{a}	35.9^{b}	11.8^{c}	17.1

abc: means in the same row with different superscripts are significantly (P<0.05) different.
SEM: Standard Error of Mean; NFE:Nitrogen Free Extract; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin.

5. Conclusion

The study has shown that growth indices; such as body weight gain, feed conversion ratio as well as crude protein digestibility of male goats were depressed by aflatoxin. The feed intake required to gain unit weight, increased with increase in the aflatoxin level in the diets, which is an indication of nutrient utilization inhibition by the toxin. The inhibition of nutrient utilisation was not due to poor digestibility but possibly absorption and assimilation, because digestibility of some nutrient by animals on aflatoxin based diets were similar to animals fed control diet.

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