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Effect of supplementing sodium bentonite or activated charcoal on mineral balances in growing male goats receiving diets with or without added aflatoxin B_1

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ABSTRACT

Use of nutritionally inert sorbents like sodium bentonite (NaB), activated charocoal (AC) offers a promising approach to tackle the aflatoxin problem. The adsorbents sequester the aflatoxins in the GI tract and reduce the absorption into the body system. Growing male kids (24) weighing 11.91±0.36 kg were divided on the basis of body weight into 6 treatment groups, i.e., T_1 (green maize + concentrate mixture), T_2 (green maize + concentrate mixture supplemented with sodium bentonite (NaB) @ 2 kg/100 kg), T_3 (green maize + concentrate mixture supplemented with activated charcoal (AC) @ 2 kg per 100 kg), T_4 (T_1 + AFB_1 @ 300 ppb), T_5 (T_2 + AFB_1 @ 300 ppb), T_6 (T_3 + AFB_1 @ 300 ppb). At the end of 70 days of growth study, a metabolism trial of 6-day duration was conducted on 18 animals. Absorption and retention (% intake) of Ca was nonsignificantly lower in aflatoxin fed group (T_4) which was further reduced by the addition of AC. AFB_1 and adsorbents used in this study did not affect the P and Mg absorption and retention in kids. AC supplementation in control (T_3) as well as aflatoxin (T_6) groups resulted in low absorption and retention (% intake) of Fe than control (T_1). Lower absorption and retention of Mn observed in aflatoxin (T_4) and charcoal fed groups (T_3 and T_6) in comparison to control (T_1). P and Mg retention was not affected in statistical terms by AFB_1 or adsorbents. However, Ca, Fe, Zn and Mn retention reduced by either AFB_1 or AC or both. Hence, long term effect of using adsorbents on mineral utilization needs to be considered.

Key words: Aflatoxin, Adsorbents, Activated charcoal, Goats, Sodium bentonite

Aflatoxins (a closely related group of polysubstituted bisfuranocoumarins) are secondary fungal metabolites produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* molds. Of the aflatoxins, aflatoxin B_1 (AF B_1) has generated much concern due to its carcinogenicity, mutagenicity, and teratogenicity (Smela *et al.* 2001, Mishra and Das 2003). Ramos *et al.* (1996) has indicated that during aflatoxicosis in lambs a severe alteration in the mineral metabolism or absorption is produced, mainly due to hepatic and renal lesions. Effect of added adsorbents like sodium bentonite (NaB), activated charcoal (AC) in growing goats with or without added aflatoxin B_1 on nutrient digestibilities (Rao and Chopra 2003), growth performance (Rao *et al.* 2004a) and on serum clinical profiles (Rao *et al.* 2004 b) has been reported. Unnikrishnan *et al.* (2005) reviewed the status of chemical residues and contaminants in milk. Recent literature suggested *Saccharomyces cerevesiae* too has a potential adsorbent effect on aflatoxin B_1 without modification in the structure of AF B_1. Madrigal-Santilla'n *et al.* 2006). In the present study, the effect of added AFB_1 and/or adsorbents such as NaB and AC on mineral utilization has been discussed.

MATERIALS AND METHODS

Male growing crossbred goats (24) of Beetal and Alpine and Beetal and Saanen (11.91±0.36 kg) were offered a basal ration consisting of a concentrate mixture [maize (*Zea mays*) grain, 25%; barley (*Hordeum vulgare*) grain 27%; mustard (*Brassica juncea*) cake (expeller), 10%; groundnut (*Arachis hypogea*) cake (expeller) 10%; wheat (*Triticum aestivum*) bran, 25%; mineral mixture, 2%; salt, 1%] and green maize for an adaptation period of 20 days. Based on their live weights the kids were divided into 6 experimental groups, consisting of 4 animals each in a randomized block design. The experimental groups comprised T_1 (green maize + concentrate mixture), T_2 (green maize + concentrate mixture supplemented with NaB @ 2 kg/100 kg), T_3 (green maize + concentrate mixture supplemented with AC @ 2 kg per 100 kg), T_4 (T_1 + AFB_1 @ 300 ppb), T_5 (T_2 + AFB_1 @ 300 ppb), T_6 (T_3 + AFB_1 @ 300 ppb). Chaffed green maize fodder was

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offered as the sole source of roughage. The aflatoxin content of the concentrate mixture was quantified by high-pressure liquid chromatography as described by Pons *et al.* (1980) with a slight modification. 50 mg of AFB_1 was dissolved in 25 ml chloroform. Cellulose paper was used to prepare sachets. Sachets containing the AFB_1 with a pinch (~ 0.5 g) of corn starch as a carrier and carefully fed to the kids in the treatment groups (T_4, T_5 and T_6) orally using long forceps so as to avoid spillage at the same time when they were fed the concentrate. The amount of AFB_1 offered was 300 ppb of the ration, approximately 11 μg/kg body weight. A metabolism trial of 6 days was conducted on 3 animals from each group after completion of 70 days of study. The goats were properly harnessed in individual metabolic cages and given 2 days adaptation period before the actual sampling of feeds, faeces and urine. The body weights of kids were recorded before and after the metabolism trial. Samples of offered and left out feeds were collected in the morning for estimation of dry matter and minerals.

Acid extract of feed, faeces and adsorbents were used for analysis of minerals Ca, P, Mg, Zn, Fe and Mn using atomic absorption spectrophotometer (acetylene as fuel and air as oxidant). Urine samples were wet digested with tri-acid mixture. Five ml urine sample was taken in a 100 ml Kjeldahl flask to which equal volume of tri acid mixture was added. The contents were made to 25 ml with distilled water. Minerals (Ca, Mg, Fe, Zn and Mn) were estimated by AAS as per cook book guide lines (AAS Data Book 1988). Total phosphorus in feed, faeces and urine samples was estimated (Ward and Johnson 1962). The differences in the treatment means between different treatment groups were analyzed for significance by one-way analysis of variance in a completely randomized design (Snedecor and Cochran 1968).

RESULTS AND DISCUSSION

The data regarding mineral concentrations of dietary ingredients is presented in Table 1. The NaB used in the study contained higher levels of Fe (5.19%) and Mn (1342 ppm). The AC used in this study did not contain appreciable amounts of minerals. Excepting Ca (1499 ppm) and Mg (142 ppm), water used in this study contained trace amounts of other minerals.

Table 1. Mineral composition of different dietary ingredients used in the trial (DM basis)

Parameter	Concentrate Mixture	Maize (Green)	Sodium Bentonite	Activated Charcoal	Water (ppm)
Calcium (%)	1.417	0.835	0.75	0.24	1499
Phosphorus (%)	0.841	0.277	ND	0.28	6.55
Magnesium (%)	0.337	0.072	0.45	0.85	142
Zinc (ppm)	78.23	14.35	75.00	22.50	2.75
Iron (ppm)	1243.77	1351.42	5.19%	ND	3.80
Manganese (ppm)	88.62	45.68	1342.00	44.70	2.41

ND, Not detectable.

The absorption of calcium (% intake) and phosphrous in various treatment groups (Table 2) was in agreement with the reported values (Haenlein 1992, Anbalagan 1997). The absorption coefficients for Mg in the present study were somewhat higher in comparison to the values (9-54%) reported by Henry and Benz (1995) in ruminants consuming mixed concentrate and roughage diets. The higher values obtained in the present study might be due to use of cereal grains (maize and barley) and maize fodder which contains fermentable carbohydrates (starch). In the present study, NaB did not affect the absorption and retention of calcium and

Table 2. Effect of supplementing sodium NaB or AC on macro mineral balances in kids fed diets added with or without AFB_1

Parameter	Treatment particulars					
	T_1 (control)	T_2 (NaB control)	T_3 (AC control)	T_4 (control + AFB_1)	T_5 (NaB control + AFB_1)	T_6 (AC control + AFB_1)
Ca intake (g/d)	8.872±1.307	7.493±0.693	10.263±0.563	7.838±0.585	8.677±0.727	9.697±0.974
Ca absorption (% intake)*	61.17^{b}±4.84	60.20^{b}±2.42	62.40^{b}±1.61	50.00^{ab}±4.52	54.45^{ab}±4.23	41.83^{a}±4.56
Ca retention (% intake)*	59.76^{b}±5.05	58.82^{b}±2.46	60.63^{b}±1.47	48.65^{ab}±4.80	50.91^{ab}±4.25	40.33^{a}±4.44
Dietary Ca retention (%)	0.89	0.93	0.97	0.66	0.77	0.61
P intake (g/d)	3.935±0.407	3.483±0.452	4.156±0.232	3.546±0.269	3.756±0.315	4.145±0.313
P absorption (% intake)	45.10±4.46	30.56±6.13	39.76±11.36	23.31±5.55	4.79±6.30	26.74±12.92
P retention (% intake)	44.79±4.40	29.84±6.30	39.18±11.30	22.88±5.37	3.89±6.10	25.67±12.88
Dietary P retention (%)	0.30	0.23	0.26	0.14	0.03	0.17
Mg intake (g/d)	1.811±0.170	1.598±0.120	1.945±0.079	1.611±0.075	1.791±0.065	1.949±0.140
Mg absorption (% intake)	90.96±0.37	91.17±0.22	90.02±0.66	90.49±0.84	90.02±0.93	89.54±0.46
Mg retention (% intake)	88.81±0.21	89.63±0.33	88.62±0.58	89.54±0.98	87.08±1.14	86.17±0.50
Dietary Mg retention (%)	0.27	0.30	0.27	0.25	0.26	0.26

*a, b, c, d: Values bearing different superscripts in a row differ significantly ($P < 0.05$).

phosphorous in contrary to the earlier reports in lactating cows (Rindsig and Schultz 1970) and in pigs (Schell *et al.* 1993). Absorption and retention of Ca, P and Mg was not affected by addition of NaB and AC under normal feeding situations. However, when AFB_1 was administered @ 300 ppb, there was significant reduction ($P < 0.05$) in the absorption and retention of Ca. P absorption and retention was similar ($P>0.05$) in all groups eventhough there are numerical differences indicating individual variations among animals within a particular group. Mg absorption and retention was also similar ($P > 0.05$) indicating that neither AFB_1 nor adsorbent affected Mg absorption and retentions. Johri (1998) reported that calcium and phosphorus retentions were significantly improved in the birds that received AC (0.1%) along with AFB_1 (0.6 ppm). In the present study, AC was used at 1.26% of diet (DM) in comparison to lower levels (0.1%) used by Johri (1998). Prasad and Chhabra (2000) used charcoal @ 1 or 2 g/kg body weight for detoxification of pesticides in crossbred cattle. Probably, the higher levels of charcoal used in the present study might have masked the effect on mineral utilization. The calculated retentions of Ca, P and Mg as% of diet varied from 0.61 to 0.89, 0.03 to 0.30, 0.25 to 0.30 as against the dietary requirement of 0.7, 0.5 and 0.2%, respectively.

In the present study, the absorption coefficient of iron was between 32.80 and 59.44 in different treatment groups, which was similar to earlier reported value of 43.10% in growing kids (Vinod and Harjit 1987). Absorption and retention of iron (% intake) was apparently lower in aflatoxin fed kids (T_4) than that in control (T_1). NaB used in this study had high concentrations of iron which was reflected in higher balances of iron in both the bentonite supplemented groups (T_2 and T_5). AC supplementation in control (T_3) as well as aflatoxin (T_6) groups resulted in low absorption and retention (% intake) of Fe than control (T_1), however, the quantity that was retained, i.e., 296.87±87.35 and 248.99±53.12 mg/day was sufficient to meet the daily requirement of kids (Haenlein 1992). The absorption coefficient of zinc was between 31.74 and 46.60% in different treatment groups in the present study, which is in agreement with the reported (16 to 51) values (Haenlein 1992) for goats. The absorption and retention (%) of Zn in different treatment groups was similar even though there are some numerical differences.

The absorption coefficient of manganese in the present study ranged between 36.52 and 56.86% in different treatment groups. The values are similar to values (25.50 to 55.75) reported by Anbalagan (1997). Absorption and retention of Mn was significantly ($P<0.01$) higher in bentonite supplemented groups (T_2 and T_5) than that in rest of the treatments primarily due to higher Mn content of bentonite. Mn absorption and retention in bentonite supplemented groups (T_2 and T_5) when calculated as per cent of intake was similar ($P>0.01$) to control (T_1). Schell *et al.* (1993) observed that there was no effect of feeding 1% bentonite with or without aflatoxin B_1 on Mn absorption and retention in growing pigs. Lower absorption and retention of Mn observed in aflatoxin (T_4) and charcoal fed groups (T_3 and T_6) in comparison to control (T_1) implied that some detrimental effect of aflatoxin and/or charcoal on Mn utilization might be possible in growing kids (Table 3). Calculated retentions of iron, Zn and Mn (ppm) varied from 384.84 to 1391.93, 13.64 to 20.07 and 24.27 to 66.97 as against dietary requirement of 30 to 100, 10 to 50 and 20 to 40, respectively.

On perusal of results it could be seen that P and Mg retention did not get affect in statistical terms by AFB_1 or adsorbents. However, Ca, Fe, Zn and Mn retention reduced by either AFB_1 or AC or both. The results indicated that utilization of minerals was affected by administering AFB_1

Table 3. Effect of supplementing sodium NaB or AC on trace mineral balances in kids fed diets added with or without AFB_1

Parameter	Treatment particulars					
	T_1 (control)	T_2 (NaB control)	T_3 (AC control)	T_4 (Control + AFB_1)	T_5 (NaB control + AFB_1)	T_6 (AC control + AFB_1)
Fe intake (mg/d)**	$667.56^{a}\pm108.42$	$1128.44^{b}\pm87.72$	$786.95^{a}\pm69.15$	$711.81^{a}\pm72.43$	$1343.13^{b}\pm69.06$	$761.00^{a}67.73$
Fe absorption (% intake)*	$55.34^{b}\pm4.82$	$58.90^{b}\pm2.82$	$36.98^{a}\pm8.68$	$42.90^{ab}\pm3.48$	$59.44^{b}\pm5.01$	$32.80^{a}\pm4.72$
Fe retention (% intake)*	$54.57^{b}\pm4.49$	$58.54^{b}\pm2.87$	$36.23^{a}\pm8.68$	$42.47^{ab}\pm3.34$	$59.17^{b}\pm4.99$	$32.08^{a}\pm4.68$
Dietary Fe retention (ppm)	616.55	1391.93	462.00	523.64	1320.47	384.84
Zn intake (mg/d)	37.16±3.70	33.03±2.64	40.71±1.79	33.66±1.96	36.68±2.59	39.85±3.28
Zn absorption (% intake)	46.60±11.42	31.74±3.47	43.65±7.58	37.54±9.04	42.47±8.48	39.78±6.96
Zn retention (% intake)	30.11±9.97	19.00±4.69	31.70±8.69	30.68±8.28	30.23±7.74	28.78±8.38
Dietary Zn retention (ppm)	20.07	13.64	19.78	17.71	17.84	18.25
Mn intake (mg/d)**	$45.45^{a}\pm5.87$	$64.70^{bc}\pm5.25$	$49.25^{ab}\pm2.87$	$41.76^{a}\pm3.47$	$73.35^{c}\pm4.67$	$48.01^{a}\pm4.38$
Mn absorption (% intake)**	$50.67^{bc}\pm2.94$	$51.56^{bc}\pm3.28$	$44.91^{abc}\pm2.30$	$39.85^{ab}\pm3.36$	$56.86^{c}\pm3.46$	36.52a±0.77
Mn retention (% intake)**	$47.36^{bcd}\pm3.07$	$49.03^{bcd}\pm3.45$	$40.92^{abc}\pm2.36$	$36.79^{ab}\pm2.57$	$55.04^{d}\pm3.42$	$32.57^{a}\pm0.98$
Dietary Mn retention (ppm)	36.73	66.97	31.20	27.05	66.93	24.27

a, b, c, d: Values bearing different superscripts in a row differ significantly ($P < 0.05$ and **$P < 0.01$).

as well as addition of adsorbents such as NaB and AC. Hence, long term effect of using adsorbents on mineral utilization needs to be considered.

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