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Influence of Clays Addition in the Ration Containing Berseem Hay Naturally Contaminated with Aflatoxin on the Zaraibi Goats' Males Performance

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ABSTRACT

Twenty four growing Zaraibi kids, with an average age of 5-6 months and 19.25±0.51 kg weight were randomly divided into four equal groups to investigate the effect of clay addition in rations containing of neutrally contaminated and/or not Contaminated Berseem Hay (CBH) with aflatoxin. Tested Rations (R) consisted of: R1: Concentrate feed mixture (CFM)+(BH 3[rd] cut), G1, R2: CFM+CBH, G2, R3: CFM+BH+aluminosilicate, G3 and R4: CFM+CBH+ aluminosilicate, G4. The obtained results indicated that total DM intake was the lowest (809 g h^{-1}) in G2 while the highest value (850 g h^{-1}) was noticed with G3, followed by G4 (843 g h^{-1}) then G1 (833 g h^{-1}). However, Clays addition reduced the water consumption by kids. The daily body weight gain was significantly ($p<0.05$) enhanced in G3 and G4 supplemented with clay. Feed utilization was improved and the effect was more pronounced in group G3. Significant decrease ($p<0.05$) in blood serum creatinine, cholesterol and ALT concentrations in G4 compared with G2 was detected. Significant differences ($p<0.05$) in hemoglobin, MCHC and lymphocytes concentration between G4 and G2 were recorded. No significant differences were detected in respiration rate, pulse and rectum, skin temperature values among tested groups. Clays addition decreased the incidence of soft feces, bloating and diarrhea in group G3 and G4. Clays addition improved economic efficiency. Accordingly, it could be concluded that clays addition improved growth performance, liver and kidney function, as well as the economic efficiency with growing Zaraiby kids.

Key words: Zaraibi kids, aflatoxin, clays, growth performance, digestive and blood parameters

INTRODUCTION

Aflatoxins are toxic secondary metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The most well-known aflatoxins are B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is the most common while it can be detected in feedstuffs in quite high concentrations compared with the other aflatoxins (Kourousekos *et al*., 2012). Contamination with mycotoxins often begins in the field and increases during harvest, drying and storage (CAST., 2003). Two primary factors influencing mycotoxin production both pre-harvest and post-harvest are temperature and moisture.

The aflatoxins cause significant economic losses in animals due to reduced productivity, increased disease incidence, chronic damage of vital organs and decreased reproductive performance (Iheshiulor *et al*., 2011). Besides chronic exposure compromises immunity and interferes with protein metabolism and metabolism of micronutrients (Schell *et al*., 1993; Marin *et al*., 2002).

Several strategies for the decontamination/detoxification of grains contaminated by mycotoxins have been reported using physical, chemical and biological methods specific to the commodity (Upadhaya *et al*., 2009). One of the more effective and practical approaches is the use of adsorbents. Adsorbents added to aflatoxin-contaminated feeds can sequester aflatoxin during the digestive process, allowing aflatoxin to pass harmlessly through the animal (Phillips, 1999). However, not all adsorbents are equally effective at sequestering aflatoxin (Stroud, 2006) and some may interfere with nutrient utilization.

However, several studies have demonstrated that bentonite and other such feed additives can effectively reduce or prevent the toxicity caused by feed contaminated with *Aspergillus* mycotoxins, such as AfB1 (Trckova *et al*., 2004; Kannewischer, 2006; Jaynes and Zartman, 2011). Moreover, it is stated that bentonites in animal diets act as gut protectants (enterosorbents) and that bentonites have been shown to be the most effective in binding aflatoxin (Trckova *et al*., 2004).

The main objectives of the present study were to investigate the effect of the addition of clays in the ration naturally contaminated with aflatoxin on the growth performance, blood biochemical, hematological and physiological parameters of Zaraibi goats' males and on the economic efficiency. Some digestive and metabolic disturbances were also studied.

MATERIALS AND METHODS

This study was conducted at El-Serw Experimental Station belonging to the Animal Production Research Institute, Agriculture Research Center, Giza, in cooperation with Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt. Twenty four growing Zaraibi kids, selected from El-Serw Station Herd, with an average age of 5-6 months and 19.25±0.51 kg weight were used. The animals were randomly divided into four equal groups (6 of each) according live body weight. Each group was housed in a semi-roofed yard (4×3×5 m) and was randomly fed one of the following tested rations (R):

- **R1:** Concentrate feed mixture (CFM) +berseem hay (BH 3rd cut), G1
- **R2:** CFM+CBH (contaminated berseem hay), G2
- **R3:** CFM+BH+ aluminosilicate, G3
- **R4:** CFM+CBH + aluminosilicate, G4

Feed intake and body weigh changes of animals were biweekly adjusted according to the body weigh changes. Kids were fed for 2 weeks as a transitional period on the experimental rations before starting the experimental work. The feeding trails lasted for 16 weeks and animals were raised under hygienic and managerial conditions.

Kids were offered their requirements of CFM and BH according to NRC (1981) allowances of male Zaraibi goats (kids) and the amounts of CFM and BH were estimated to cover 60 and 40 of dry matter requirements, respectively. The CFM consisted of 26% un-decorticated cotton meal, 40% yellow corn, 26% wheat bran, 4% molasses, 2.5% limestone, 1% common salt and 0.5% minerals mixture. The BH used in tested rations (R2 and R4) was either non or naturally contaminated during the storage period at the station. Representative samples were taken from BH and assay of aflatoxin was done according to Shannon *et al*. (1983). The contaminated BH contained

Table 1: Chemical composition of feed ingredients (%, DM)

Items	DM	Chemical composition (%)					
		OM	CF	CP	EE	NFE	Ash
Concentrate feed mixture (CFM)	91.30	93.95	15.85	14.50	3.30	60.30	6.05
Uncontaminated berseem hay (UBH)	90.05	87.89	28.95	11.20	2.15	45.59	12.11
Contaminated berseem hay (CBH)	87.30	87.59	29.06	11.29	2.27	44.97	12.41

67.2 ppb mixture of four types of aflatoxin being 29.9 ppb AFB1, 0.70 ppb AFB2, 35.7 ppb AFG1, 0.90 ppb AFG2 mg kg^{-1} DM but the ration R1 and R3 were uncontaminated and free of aflatoxin. The chemical composition of the CFM and BH is presented in Table 1. Aluminosilicate clay was bought from Sina Co. for Phosphate and Manganese (Fayoum Governorate, Egypt). It was mixed thoroughly with some ground CFM and fed with it in ration of G3 and G4 at the rate of 1.5% of the total daily feed intake.

The clay level was determined based on the findings of Ahmed *et al.* (2002). The animals were fed in group feeding and the rations were offered twice daily in two equal portions at 8 am and 4 pm. Feed refusals (if any) were daily collected just before offering the next day feed. Fresh water was available all times. Some digestive disturbances (soft feces, bloating and diarrhea) were recorded.

Blood samples were collected at 2 h before morning feeding by jugular vein puncture from 3 kids of each treatment at the end of the tested groups. The blood serum was separated and stored at -20°C until analysis. Serum Total Protein (TP) was measured by the Biuret method according to Henry *et al.* (1974), cholesterol according to Pisani *et al.* (1995). Albumin concentration was determined according to the method of Doumas *et al.* (1971) while globulin was calculated. Liver function was assessed by measuring the activities of aspartates aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) in the serum as described by Reitman and Frankel (1957). Kidney function was evaluated by measuring blood urea using the colorimetric methods according to Henry and Todd (1974) using commercial kits. Creatinine was measured using the colorimetric method according to Faulkner and King (1976). Representative samples of feed ingredients were analyzed for summative analysis according to AOAC (1995).

Statistical analysis: Data was statistically analyzed using the General linear Model (SAS., 2008) for complete randomized design. The significant differences among means were assigned using Duncan multiple range test method (Duncan, 1955).

RESULTS

The data relevant to feed intake and water consumption by kids are presented in Table 2. The obtained results indicated that total DM intake (g h^{-1}) was the highest in G3 fed BH+ aluminosilicate followed by G4 fed CBH+ aluminosilicate and the lowest value was noticed with G2. However, DM intake expressed as % BW or g/$kg^{w\ 0.75}$ was practically similar in all treatments. Total CP intake was slightly lower in G2 fed contaminated berseem hay than other groups. The ratios of roughage to concentrate (R/C) were round 40:60 in the all groups, except G2 was 38:62.

The obtained results showed the role of clays addition in reducing the water consumption by kids. The highest values of all parameters of water consumption were observed with G2 fed CBH while the lowest values were recorded with G3 which fed BH+aluminosilicate.

Results of growth performance of male Zaraibi goats fed experimental rations are shown in Table 3. As can be seen that the values of final Live Body Weight (LBW), Total Body Gain (TBG) and Daily Body Gain (DBG) were significantly ($p<0.05$) enhanced in G3 and G4 supplemented with clay, compared with those of G1 and G2. Results in Table 3 also showed that feed utilization expressed as kg DM/kg gain and kg CP/kg gain were improved by clays supplementation and such effect was more pronounced with non-contaminated BH (G3) group compared with contaminated one (G4).

Table 2: Daily dry matter intake and water consumption by male Zaraibi goats

	Groups			
Items	G1	G2	G3	G4
Daily feed intake (g/head)				
From CFM	503	498	507	505
From BH	330	311	343	338
Total DM intake	833	809	850	843
Total CP intake	110	107	112	111
DM intake, % BW	3.52	3.55	3.49	3.51
DM intake, g/kg$^{w\ 0.75}$	77.63	77.56	77.48	77.70
Roughage: Concentrate (R/C) ratio	40:60	38:62	40:60	40:60
Water consumption				
L/head/day	2.53	2.75	2.41	2.60
mL/kg BW	107	121	98.8	108
mL/kg$^{w\ 0.75}$	236	264	220	240
mL/g DM intake	3.04	3.40	2.84	3.08

Table 3: Effect of feeding rations on growth performance and biochemical parameters of male Zaraibi kids

	Groups			
Items	G1	G2	G3	G4
Growth performance				
Initial LBW (kg)	19.20±1.45	18.80±1.35	19.60±1.00	19.40±1.20
Final LBW (kg)	28.15±0.68^{a}	26.75±0.78^{b}	29.18±0.76^{c}	28.62±0.84^{ac}
Total Body Gain (TBG) (kg)	8.95±1.00^{a}	7.95±0.73^{b}	9.58±0.37^{a}	9.22±0.49^{a}
Daily Body Gain (DBG) (g)	79.92±8.96^{a}	70.98±6.53^{b}	85.57±3.26^{a}	82.30±4.34^{a}
Feed utilization				
Kg DM/kg gain	10.42	11.40	9.93	10.24
Kg CP/kg gain	1.38	1.51	1.31	1.35
Liver function				
Total protein (TP) (g dL^{-1})	6.50±0.20	6.40±0.44	6.67±0.23	6.53±0.21
Albumin (A) (g dL^{-1})	3.03±0.29	3.10±0.20	3.07±0.21	3.00±0.10
Globulin (G) (g dL^{-1})	3.47±0.15^{ab}	3.30±0.10^{b}	3.60±0.17^{a}	3.53±0.15^{ab}
A/G ratio	0.88±0.11	0.94±0.09	0.85±0.08	0.85±0.04
Glucose (mg dL^{-1})	55.33±2.52	54.00±1.73	55.67±2.52	56.00±2.65
Cholesterol (mg dL^{-1})	90.00±2.65^{ab}	94.67±3.21^{a}	85.00±2.65^{b}	87.00±3.00^{b}
AST (U L^{-1})	58.00±5.29	61.00±5.29	55.33±4.93	56.67±4.62
ALT (U L^{-1})	15.33±0.76^{b}	17.76±0.76^{a}	14.00±0.44^{c}	15.17±0.42^{b}
ALP (U L^{-1})	119.00±3.46^{ab}	125.30±6.66^{a}	113.00±5.29^{b}	115.00±5.29^{ab}
Kidney function				
Urea-N (mg dL^{-1})	15.90±0.95^{b}	17.70±0.92^{a}	15.30±0.85^{b}	16.10±0.90^{ab}
Creatinine (mg dL^{-1})	0.85±0.02^{b}	0.93±0.03^{a}	0.80±0.04^{b}	0.86±0.04^{b}

$^{a\text{-}c}$Means in the same row with different superscripts are significantly different at $p<0.05$

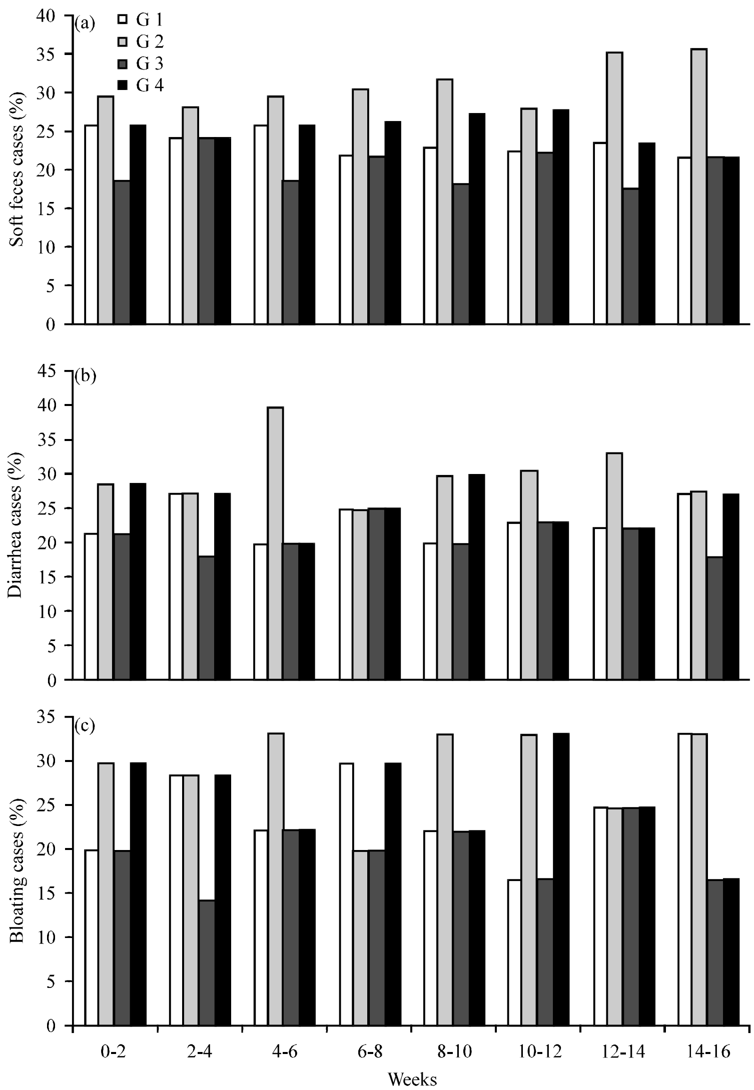

Fig. 1(a-c): Incidence of (a) Soft faces, (b) Diarrhea and (c) Bloating in growing Zaraibi kids

Data in Table 3 and 4 shows the effect of dietary treatments on some blood serum biochemical parameters studied. It could be noticed that AF increased most of blood serum parameters but no significant changes were detected. While Table 1 showed that the supplementation positively affected significantly some hematological parameters. There were significant differences in hemoglobin, MCHC and lymphocytes between G4 and G2.

Table 4 presents the effect of feeding rations on some tested physiological parameters. The results indicated no significant differences in respiration rate, pulse and rectum, skin temperature values were detected among tested groups and the animals, generally were in good health conditions.

Results in Table 5 and Fig. 1a-c showed the frequency of digestive disturbance cases. It was noticed that G2 fed CBH showed the highest values of digestive disturbance cases. Clay addition decreased the incidence of soft feces, bloating and diarrhea in group G3 and G4 but such effect was more pronounced with G3 compared with G4.

Table 4: Effect of feeding ration on blood hematological parameters and physiological parameters of growing Zaraibi kids

	Groups			
Items	G1	G2	G3	G4
Hematological parameters				
Hemoglobin (Hb) (g dL^{-1})	11.60±0.44[ab]	11.03±0.31[b]	12.23±0.31[a]	11.80±0.44[a]
Hematocrit (Hct) (%)	34.07±1.25	33.93±1.29	34.00±1.80	33.43±1.80
Red blood cells (RBCs) (10^6 μL^{-1})	13.53±1.00	13.00±0.56	14.03±0.55	13.67±0.76
Mean cell volume (MCV) fL	20.17±1.26	19.33±1.26	20.67±1.26	19.97±0.90
Mean cell hemoglobin (MCH) Pg	5.77±0.64	5.60±0.66	6.03±0.50	5.90±0.69
Mean cell hemoglobin concentration (MCHC) (%)	34.02±1.04[b]	32.50±0.35[c]	35.77±0.35[a]	35.30±0.36[a]
White blood cells (WBCs) (10^3 μL^{-1})	9.90±0.40[ab]	10.47±0.40[a]	9.60±0.36[b]	9.73±0.45[ab]
Lymphocytes (%)	59.70±0.75[b]	57.27±0.75[c]	61.47±0.61[a]	60.63±1.19[ab]
Neutrophils (%)	35.50±1.45	36.80±1.97	34.53±1.69	35.27±1.57
Monocytes (%)	4.60±0.79	5.10±1.11	4.00±1.08	4.03±0.50
Physiological parameters				
Respiration rate	26.00±3.58	27.33±5.47	25.50±2.35	26.50±3.08
Pulse	84.50±4.64	86.00±7.87	85.17±2.79	85.50±5.13
Rectum temperature	39.17±0.22	39.28±0.34	39.13±0.16	39.15±0.15
Skin temperature	38.07±0.40	38.15±0.58	38.08±0.41	38.10±0.42

[a-c]Means in the same row with different superscripts are significantly different at $p<0.05$

Table 5: Effect of feeding rations on frequency of digestive disturbances cases in growing Zaraibi kids

	Groups			
Items	G1	G2	G3	G4
Soft Feces incidence (%)	23.44	31.04	20.28	25.24
Diarrhea incidence (%)	23.28	30.28	21.01	25.43
Bloating incidence (%)	24.75	29.61	19.63	26.00

Table 6: Effect of feeding experimental rations on economic efficiency of kids

	Groups			
Items	G1	G2	G3	G4
Daily body gain (g h^{-1})	79.92	70.98	85.57	82.30
Daily feed intake (g h^{-1})				
From CFM	551.00	545.00	555.00	553.00
From BH	366.00	356.00	381.00	387.00
From silicate	-	-	14.00	14.00
Cost of consumed feed (pt h^{-1})	146.80	144.60	149.80	150.00
Price of weight gain (pt h^{-1})	240.00	213.00	257.00	247.00
Feed cost/kg gain (LE)	18.35	20.37	17.52	18.23
Economic efficiency (%)	1.63	1.47	1.72	1.65

Prevailing price per ton at the time of study was 2000 LE for CFM, 1000 LE for BH and 500 LE for clay, while selling prices of 1 kg LBW was 30 LE

The highest total feed cost/kg gain (Table 6) along the feeding period was observed for G2 while the intermediate values were recorded for G1 and G4 and the lowest value was for G3, due to the highest daily body gain as well as the highest price of weight gain in this group (G3). Economic efficiency values revealed that G3 had the highest economical feed efficiency, followed by G4 and G1 and lastly G2.

DISCUSSION

This study was conducted to investigate the effects of clay addition in rations containing of neutrally contaminated and/or not contaminated berseem hay with alflatoxin. The obtained results showed the role of clays addition in increasing DMI and reducing the water consumption by Zaraibi kids. Moldy diets decreased the feed intake and increased the water consumption with lactating ewes (Hassan, 2003) and rabbits (Abdelhamid, 2009) have been reported. Water consumption reduction could be related to the increase of kidney function to remove the AF effect (Hassan, 2003).

Clay addition in present study improved LBW, TBG and DBG and feed utilization and this may be due to the role of clays on increasing digestibility and absorption of nutrients (Nowar *et al.*, 1993). Similar trend on goats was observed by Mousa (1996) and Abdelhamid *et al.* (1999). However, lower growth performance in groups fed contaminated BH is possibly the most obvious indication for chronic aflatoxicosis and other mycotoxicoses (Pier, 1992) and is could be related to disturbances in protein, carbohydrate and lipid metabolism (Cheeke and Shull, 1985). Similarly, increasing AF in cattle feed at different levels has been shown to significantly reduce feed intake at all different levels in a dose-dependent manner (Choudhary *et al.*, 1998). In a 155 day feeding trial, AFB1 was shown to depress feed efficiency and rate of gain in steers (Helferich *et al.*, 1986). Contrarily, levels of AF at feed offered to weanling goats for up to 112 day had no effects in terms of DMI, apparent nutrient digestibilities, or body weight gain and did not show any noticeable signs of toxic effects (Gurung *et al.*, 1998). However, decreased feed efficiency in cattle has been attributed to compromised ruminal function by reducing cellulose digestion, volatile fatty acid production and rumen motility (Helferich *et al.*, 1986; Diekman and Green, 1992).

It could be noticed in the present study that AF increased most of blood serum parameters but no significant changes were detected. These findings may lead to the assumption that aflatoxin had a slight effect on hepatic cells but not to an extent that the hepatic function could be affected. On the other hand, the results manifested a significant decrease in creatinine, cholesterol and ALT concentrations in the groups fed BH supplemented with clays. In this respect, Abdelhamid (2009) reported that Ochratoxicosis-a by rabbits reflected increases in concentrations of blood creatinine, urea, uric acid, cholesterol and transaminases activity, whereas decrease of blood TP concentration. In the same aspect, Kourousekos *et al.* (2012) stated that the consumption of AFB1 by the goats increased significantly the AST concentrations in the experimental groups. Bingol *et al.* (2007) reported that, on goats there was a significant negative correlation between total protein levels, albumin, globulin and ALT concentration and AF in feed. Moreover, Battacone *et al.* (2003) administered three different doses of AFB1 in ewes and observed a significant effect on ALT and ALP concentrations. Additionally, Gurung *et al.* (1998) showed that AF increased concentrations of cholesterol in exposed goats.

On the same hand, Clark *et al.* (1984) showed with goats that AST values were increased significantly in all groups and this increase was dose dependent, leading the authors to consider AST as a good indicator of aflatoxicosis. On the contrary, ALT and ALP concentrations of the 3 experimental groups differed considerably from those of controls, presenting lower values (Clark *et al.*, 1984). Increased AST activity after aflatoxin administration in goats was also observed by Maryamma and Sivadas (1975). In this field, Donmez *et al.* (2012) showed that erythrocyte count, leukocyte count, hemoglobin and hematocrit levels were decreased in AF group of rams. However, aflatoxicosis caused the lymphocytopenia and monocytopenia but increased percentage of neutrophil counts (Donmez *et al.*, 2012). In another study (Fernandez *et al.*, 1997), lambs fed

AF of feed daily for 21 days showed symptoms of clinical aflatoxicosis including hepatic and nephritic lesions, altered mineral metabolism and increased size and weight of the liver and kidney.

A further interesting finding of the present study was the Clays addition decreased the incidence of soft feces, bloating and diarrhea in the groups fed CBH. While, no significant differences were detected in respiration rate, pulse and rectum, skin temperature values among tested groups. However, animal feeding studies have demonstrated that bentonite and other such feed additives can effectively reduce or prevent the toxicity caused by feed contaminated with *Aspergillus* mycotoxins, such as AfB1 (Bailey *et al.*, 2006; Fairchild *et al.*, 2008; Magnoli *et al.*, 2008). Abdelhamid (2009) reported that Ochratoxicosis-a by rabbits reflected clinical symptoms including feed refusal, increased pulse rate, hard breathing, land lay and slack. Also, the post-mortem examination showed presence of blue spots on the lungs, hemorrhagic patches on the esophagus, stomach, intestine, heart, kidneys and dilated kidneys (Abdelhamid, 2009). Moreover, these results are in accordance with those obtained by Abdelhamid *et al.* (1999). Dietary clays inclusion reduced the incidence of dyspepsia (indigestion) in offspring and mature goats (Abdelhamid *et al.*, 1999) and significantly reduced bloat score (Carruthers, 1985). Also, Abdelhamid (2009) reported that Aflatoxicosis by rabbits characterized by felt fur, high body temperature, loss of appetite, weakness, bloody diarrhea, nervous movements, paralysis and death.

Moreover, in this study, clays addition improved economic efficiency. In the same trend, positive effect of bentonite inclusion in animal diets on the economic efficiency with goats (Abdelhamid *et al.*, 1999) and growing sheep (El-Saadany *et al.*, 2003) have been reported.

CONCLUSION

From the foregoing results, it could be concluded that an aflatoxin binder (e.g., aluminosilicate clays) should be added to the feed roughages whenever aflatoxin infected feed is being utilized in feeding animals or whenever mycotoxins are suspected. Moreover, beneficial positive effects were obtained in most of tested parameters as a result of adding clays to un-infected roughage and hopefully more research work is required in future to clarify this point. Constant monitoring on farm feed management, including of mould inhibitors and binders, will reduce the hazardous economic impact of moulds and mycotoxins on animal performance.

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