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RESEARCH ARTICLE

Impacts of bentonite supplementation on growth, carcass traits, nutrient digestibility, and histopathology of certain organs of rabbits fed diet naturally contaminated with aflatoxin

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Abstract The present study was conducted to investigate the effect of graded levels of dietary bentonite supplementation on growth performance, carcass traits, nutrient digestibility, and histopathology of certain organs in rabbits fed a diet naturally contaminated with aflatoxin. In total, 125 weanling New Zealand White male rabbits were randomly assigned to five treatment groups each of five replicates. Treatments were as follows: T1, basal diet with no aflatoxin and no additives (positive control diet, PCD); T2, basal diet naturally contaminated with 150 ppb aflatoxin and no additives (negative control diet, NCD); T3, NCD plus 0.5% Egyptian bentonite; T4, NCD plus 1% Egyptian bentonite; and T5, NCD plus 1% Egyptian bentonite. The experiment lasted for 8 weeks. Results showed a significant decrease ($P < 0.05$) in the body weight and the body weight gain in the NCD, while they were improved ($P < 0.05$) in groups fed diets supplemented with different levels of bentonite. The relative weight of the liver and kidneys were higher in the NCD, while the liver weight was relatively high in the group fed NCD supplemented with 0.5% bentonite, and it was not significant in other bentonite-supplemented groups. Bentonite supplementation improved the digestibility coefficients of various nutrients. Bentonite addition decreased the histopathological lesions in liver, kidney, and intestine caused by aflatoxin-infected diets. In conclusion, bentonite supplementation overcame the negative effect of aflatoxin, enhanced growth performance traits, decreased the relative weights of the liver and the kidney which are usually increased by aflatoxin, caused significant improvement in nutrients' digestibility, and decreased the histopathological lesions caused by aflatoxin-infected diets. The level of 2% bentonite is recommended for ameliorating the aflatoxin effects.

Keywords Rabbit · Bentonite · Growth performance · Carcass · Histopathology · Aflatoxin

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Introduction

Toxication is one of the important problems which occur because of unsuitable storage of food and feedstuff; this process is usually caused by mycotoxins which are produced by mold as reported by Çelik et al. (2000). The most seen mycotoxins are aflatoxins. The most harmful type is aflatoxin B1 which is produced by *Aspergillus parasiticus* and *Aspergillus flavus* (Abdel-Wahhab et al. 2002; Eraslan et al. 2004). Contamination by aflatoxins causes harmful health challenges and losses in the production of livestock through inducing mortality and morbidity (Van Rensburg et al. 2006). Besides, existence of aflatoxins may cause pathological lesions in liver and consequently impair antioxidant functions of liver as mentioned by Yang et al. (2012). A mycotoxin-contaminated diet may result in feed refusal, poor feed conversion, decreased body weight gain, and immune suppression (CAST 1989; Lindemann et al. 1993; Kubena et al. 1998), resulting in great economical losses. The use of adsorbing agents has gained much attention to cope with aflatoxin because of its ability to trap aflatoxin molecules by the use of ion exchange and then hindering its absorption from the gastrointestinal tract into systemic blood. Zeolite (Harvey et al.

1991), bentonite (Santurio et al. 1999), hydrated sodium calcium aluminosilicate (HSCAS) (Huff et al. 1992), inorganic sorbents (Bailey et al. 1998), blend of organic acids, and activated charcoal (Edrington et al. 1997) have been reported to prevent aflatoxicosis. Bentonite (BNT) is defined as adsorbent aluminum phyllosilicate which mainly consisted of montmorillonite (MMT). Bentonites are in general classified as calcium, sodium, or mixed types, depending mainly on the dominant exchangeable ion as explained by Hassan and Abdel-Khalek (1998). Egyptian BNT which contains 60–90% montmorillonite can be used to reduce aflatoxin toxicity and enhance animal performance (Shehata 2002). Besides, the ability to use it in several medical applications has been reported (Veniale et al. 2007). Many studies assured that using silicate minerals in diets of broilers could enhance growth performance (Santurio et al. 1999; Tauqir et al. 2001; Miles and Henry 2007). Moreover, animal feed involving MMT promotes growth performance and decreases gut bacterial colonization as well as the detrimental impacts of mycotoxin-contaminated diets (Tauqir and Nawaz 2001). Miazzo et al. (2005) demonstrated that dietary supplementation of Na-bentonite may ameliorate aflatoxicosis in broilers. Magnoli et al. (2011) found that sodium bentonite can be successfully used to prevent aflatoxicosis in broiler chickens. There are limited studies on the effect of bentonite clay on aflatoxins present in diets of rabbits. Therefore, the main objective of the present study is to evaluate the effect of different levels of bentonite on growth performance, carcass traits, digestibility, and histological examination of different organs of rabbits fed diets naturally contaminated with aflatoxins.

Materials and methods

Experimental design and diets

The present study was performed at the Rabbit Research Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. All procedures of the experiment were performed with reference to the Committee of Local Experimental Animal Care and approved by ethics of our Nutrition and Clinical Nutrition Department institutional committee, Veterinary Medicine College, University of Zagazig, Egypt.

In total, 125 weanling New Zealand White (NZW) male rabbits were obtained from a commercial rabbit producer with average body weight of 0.908 ± 0.018. Rabbits were submitted to a 7-day adaptation period before the beginning of the trial. Rabbits were randomly assigned to five treatment groups each of five replicates in a complete randomized design experiment. Treatments were as follows: T1, basal diet with no aflatoxin and no additives (positive control diet, PCD); T2, basal diet naturally contaminated with 150 ppb aflatoxin and no additives (negative control diet, NCD); T3, NCD plus 0.5% Egyptian bentonite; T4, NCD plus 1% Egyptian bentonite; and T5, NCD plus 1% Egyptian bentonite. The experiment lasted for 8 weeks and fresh water was available all the time. Animals were housed in individual cages under the same managerial, hygienic, and environmental conditions all over the experimental period. The formulation and chemical composition of the basal diet are shown in Table 1. The proximate chemical analysis of the used feedstuffs and the experimental diets was carried out according to the standard procedures of the AOAC (2002).

Aflatoxin quantification and diet preparation

A complete analysis was performed for feed ingredients individually as well as screening for the content of aflatoxin. According to Romer (1975), aflatoxin was extracted and then quantified using thin-layer chromatography (TLC). Basal diet did not involve any detectable aflatoxin concentrations (below 1 μg/kg diet; ppb). From a private feed mill, corn already contaminated with mold was obtained. Corn was stored in a humid place (20% moisture)

Table 1 The proximate chemical composition of the basal diet (%)

Ingredients	%
Berseem Hay	55.80
Ground Corn	6.80
Barley Grain	34.23
Wheat bran	0.15
Soybean meal	1.20
$CaCO_3$	0.92
Premix*	0.35
NaCl	0.30
Chemical composition (%)	
Dry matter	90.05
Digestible energy (kcal/kg diet)	2506
Crude protein	16.58
Crude fat	2.57
Crude fiber	12.75
Calcium	0.62
Available phosphorous	0.52
Cystine	0.30
Lysine	0.89
Methionine	0.22

* Supplied per kilogram of diet: 12,000 IU vitamin A (7200 μg β-carotene), 55 μg of cholecalciferol, 10 IU vitamin E (10 mg of DL-α-tocopheryl acetate), 2.0 mg vitamin K_3, 1.0 mg vitamin B_1, 4.0 mg vitamin B_2, 1.5 mg vitamin B_6, 0.0010 mg vitamin B_{12}, 6.7 mg vitamin PP, 6.67 mg vitamin B_5, 0.07 mg vitamin B_8, 1.67 mg vitamin B_9, 400 mg choline chloride, 133.4 mg Mg, 25.0 mg Fe, 22.3 mg Zn, 10.0 mg Mn, 1.67 mg Cu, 0.25 mg I, and 0.033 mg Se

Table 2 The effect of dietary supplementation of different levels of bentonite on the growth performance of rabbits

Parameters	PCD	NCD	NCD + 0.5% bentonite	NCD + 1% bentonite	NCD + 2% bentonite
Initial BW (kg)	0.84 ± 0.12a	0.87 ± 0.02a	0.94 ± 0.01a	0.94 ± 0.06a	0.89 ± 0.02a
Final BW(kg)	2.21 ± 0.07ab	1.66 ± 0.04c	2.36 ± 0.04a	2.18 ± 2.04b	2.15 ± 0.04b
BWG (kg)	1.37 ± 0.07ab	0.78 ± 0.03c	1.42 ± 0.03a	1.23 ± 0.03ab	1.25 ± 0.02ab
FI (kg)	4.31 ± 0.13b	4.31 ± 0.09b	4.70 ± 0.04a	4.61 ± 0.09ab	4.52 ± 0.11ab
FCR (g feed/g gain)	3.17 ± 0.17c	5.53 ± 0.23a	3.31 ± 0.05c	3.74 ± 0.12b	3.59 ± 0.03ab
PER	1.93 ± 0.11a	1.10 ± 0.04d	1.83 ± 0.03ab	1.62 ± 0.05c	1.68 ± 0.02bc
RGR (%)	70.27 ± 2.69a	45.67 ± 1.77c	66.65 ± 7.47ab	61.23 ± 3.07b	63.66 ± 3.45ab

Means within the same row carrying different letters are significantly different at $P < 0.05$

BW body weight, *BWG* body weight gain, *FI* feed intake, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *RGR* relative growth rate

for 2 months to stimulate growth of mold. The existence of aflatoxin in corn was assured by TLC. In formulating contaminated-diet treatments, aflatoxin-free corn was replaced by naturally contaminated corn. Samples were selected randomly from four different parts of the whole sample as described by Azizpour and Moghadam (2015). Analyzing the contaminated diet revealed the presence of 150 ppb aflatoxin (the detection limit was 1 ppb). Aflatoxin in the contaminated diet consisted of 9.20% AFG1, 3.58% AFG2, 80.72% AFB1, and 6.50% AFB2.

Throughout the whole experimental period, samples of control and contaminated diets were tested for the concentration of aflatoxin and any other kind of mycotoxins. Concentration of aflatoxin in PCD was below the limits of detection. Levels of aflatoxin in the contaminated diets averaged from 140 to150 ppb.

Investigated measurements

Growth performance

Body weights (BWs) were recorded at the beginning and at the end of the experiment and feed consumption measured weekly. Body weight gain (BWG) was calculated as W2 − W1 where W1 is the initial live weight (g) and W2 is the final live weight (g). Feed conversion ratio (FCR) was estimated according to Wagner et al. (1983) as follows: FCR = amount of feed consumed (g)/body weight gain (g).

Relative growth rate (RGR) was calculated using the equation described by Brody (1968).

$$\mathrm{RGR} = \mathrm{W2\text{-}W1} / {}^{1}/_{2}(\mathrm{W1} + \mathrm{W2}) \times 100$$

Protein efficiency ratio (PER) was determined according to Mc Donald et al. (1987) as the number of grams of weight gain produced per unit of weight of dietary protein consumed.

$$\mathrm{PER} = \text{live weight gain (g)} / \text{protein intake (g)}.$$

Carcass traits

At the end of the experiment, rabbits from each group were weighed and slaughtered after 12 h of fasting. Carcasses were prepared by removing the feet, skin, genital organs, paws, digestive tract, and urinary bladder. Hot carcass weight (the main body, kidneys, head, liver, lungs, heart, and other total edible parts) were determined according to Blasco et al. (1993). Carcasses were weighed and the weights of the liver, kidneys, and heart were recorded and expressed as grams per kilogram of slaughter weight (SW). Carcass percentage = carcass weight × 100/live body weight. Dressing

Table 3 The effect of dietary supplementation of different levels of bentonite on carcass traits percent relative to the live body weight

Parameters	PCD	NCD	NCD + 0.5% bentonite	NCD + 1% bentonite	NCD + 2% bentonite
Live weight (kg)	2.06 ± 0.11a	1.72 ± 0.05b	2.17 ± 0.12a	1.94 ± 0.08ab	1.92 ± 0.12ab
Dressed weight (%)	52.42 ± 2.47a	51.88 ± 2.38 a	54.67 ± 1.25a	56.51 ± 3.35a	52.46 ± 0.85a
Liver weight (%)	3.01 ± 0.28c	6.41 ± 0 .30a	5.08 ± 0.33b	4.15 ± 0.61bc	4.09 ± 0.38bc
Kidney weight (%)	0.38 ± 0.06b	0.82 ± 0.06a	0.62 ± 0.02ab	0.46 ± 0.04b	0.41 ± 0.05b
Heart weight (%)	0.28 ± 0.03a	0.25 ± 0.02a	0.35 ± 0.05a	0.25 ± 0.01a	0.31 ± 0.04a

Means within the same row carrying different letters are significantly different at $P < 0.05$

Table 4 The effect of dietary supplementation of different levels of bentonite on the digestion coefficient of nutrients (g/kg)

Parameters	PCD	NCD	NCD + 0.5% bentonite	NCD + 1% bentonite	NCD + 2% bentonite
DM	643.33 ± 6.66c	536.66 ± 14.52d	733.33 ± 21.85ab	726.66 ± 8.81b	770.00 ± 5.77a
CP	733.33 ± 6.66b	680.66 ± 15.27c	750.00 ± 0.10b	753.33 ± 0.17b	823.33 ± 3.33a
EE	540.00 ± 17.32d	526.66 ± 12.01d	620.00 ± 10.00c	696.66 ± 3.33b	756.66 ± 6.66a
CF	357.73 ± 6.63c	363.83 ± 4.44c	477.43 ± 9.13b	460.96 ± 6.43a	467.00 ± 6.54a
Ash	460.00 ± 23.09b	433.33 ± 17.63b	510.00 ± 26.45a	576.66 ± 8.81a	665.00 ± 12.01a

Means within the same row carrying different letters are significantly different at $P < 0.05$

DM dry matter, *CP* crude protein, *EE* ether extract, *CF* crude fiber

percentage = (carcass weight plus giblet weight) × 100/live body weight.

Histopathological examination

Samples of liver, kidney, and small intestine were harvested from six birds per treatment and then fixed in 10% neutral buffered formalin for histopathology and gross evaluation. The fixed tissues of liver were trimmed, embedded in paraffin, and then sectioned at 5 μm. After this, sections were stained with eosin and hematoxylin for the microscopic examination.

Digestibility trials

At the experiment end, six rabbits per group were selected randomly for digestibility trials where the amount of feed intake and feces excreted were recorded daily. The proximate analysis of feces and feed was determined with reference to AOAC (2002).

Statistical analysis

All data were exhibited as the mean ± SD. All data were verified for normality after transformation (ASIN). One-way ANOVA was used to determine the effects of different levels of bentonite on growth performance, carcass traits, digestibility, and histopathological examination of rabbit fed a diet naturally contaminated with aflatoxin using SPSS version 17 for Windows (SPSS, Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare differences between the means at 5% probability.

Results

Growth performance

As shown in Table 2, results showed that there was a significant decrease ($P < 0.05$) in body weight and body weight gain in the NCD group. There were no significant

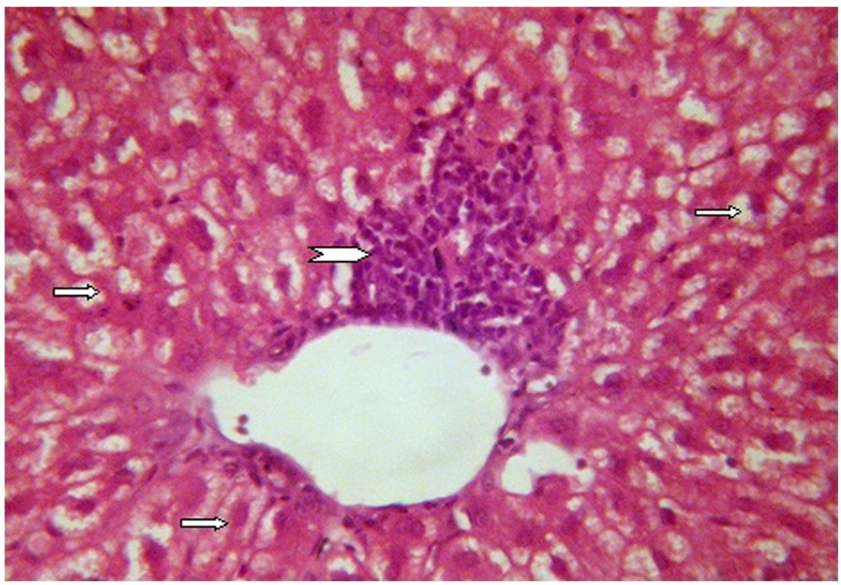

Fig. 1 The liver from the treatment NCD showing hydropic degeneration in hepatocytes (arrow) with perivascular aggregation of round cells (arrow head), HE × 1200

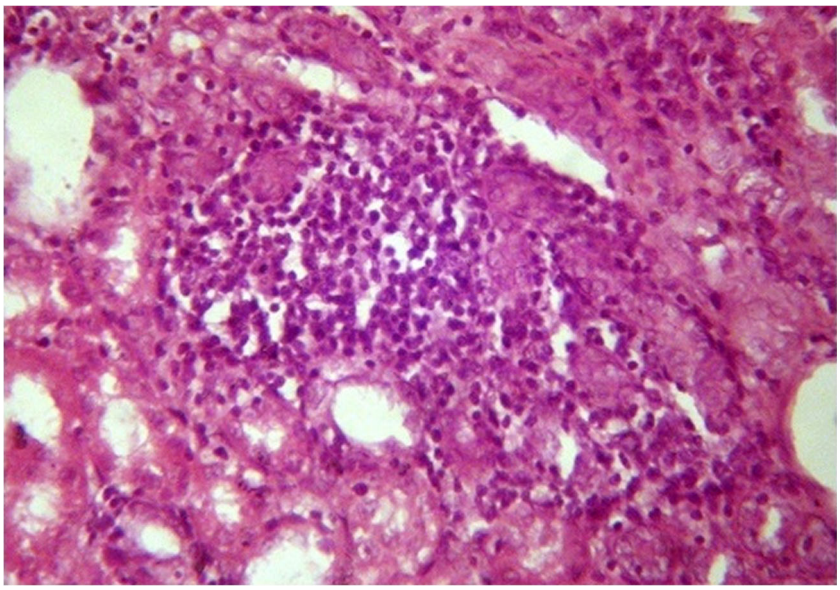

Fig. 2 The kidney from the treatment NCD showing focal aggregation of round cells replaced necrotic renal tubules (arrow), HE × 1200

differences ($P > 0.05$) in BW and BWG between PCD and the groups fed NCD supplemented with different levels of bentonite. However, the group fed NCD supplemented with 0.5% bentonite showed the best values of BW and BWG. There was no significant difference ($P > 0.05$) in feed intake (FI) between PCD and groups fed NCD supplemented with 1 and 2% bentonite, while there was statistical increase ($P < 0.05$) in FI between PCD and the group fed NCD supplemented with 0.5% bentonite. Comparing with PCD, there was a significant increase in FCR values ($P < 0.05$) in NCD and the group fed NCD supplemented with 1 and 2% bentonite. The worst FCR was found in NCD. No significant difference ($P > 0.05$) was observed between PCD and the groups fed NCD supplemented with 0.5% bentonite regarding FCR. Results showed a significant ($P < 0.05$) decrease in protein efficiency ratio (PER) in NCD and the groups fed NCD supplemented with 1 and 2% bentonite. The lowest value was found in the NCD group. Regarding relative growth rate (RGR), results showed a significant ($P < 0.05$) decrease in NCD and the groups fed NCD supplemented with 1% bentonite. The lowest value was found in the NCD group. No significant difference ($P > 0.05$) was detected in RGR between PCD and the groups fed NCD supplemented with 0.5 and 2% bentonite.

Carcass traits

Results in Table 3 showed no statistical difference ($P > 0.05$) between PCD group and groups fed NCD

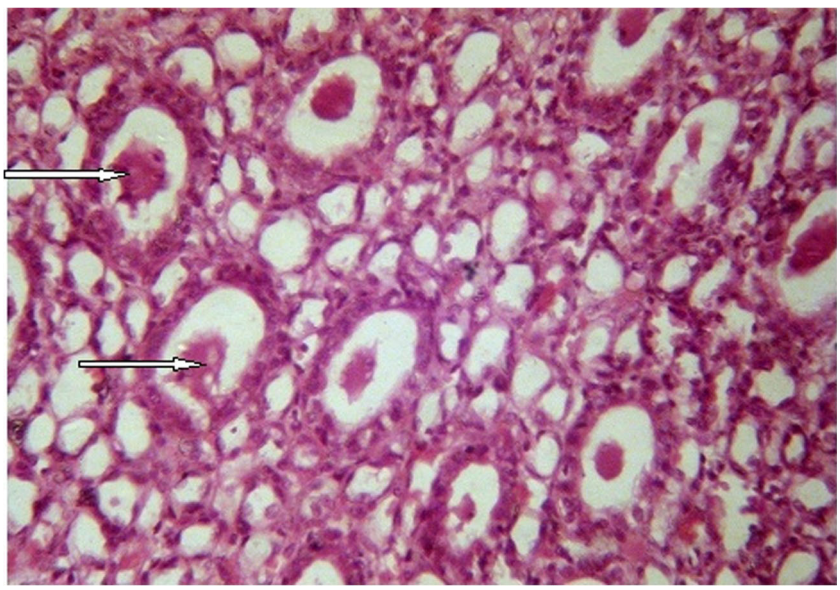

Fig. 3 The kidney from the treatment NCD showing cellular cast in the lumen of some renal tubules (arrow), HE × 1200

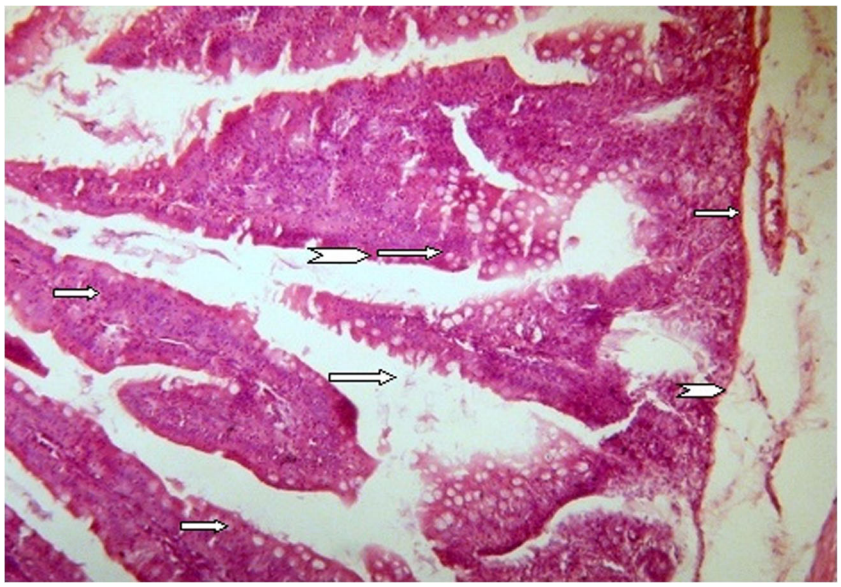

Fig. 4 the intestine from the treatment NCD showing hyperplasia and mucinous degeneration of intestinal villi (arrow) with edema in the sub-mucosa (arrow head), HE × 300

plus 1 or 2% bentonite in various carcass traits. Relative weights of liver and kidney were positively impacted ($P < 0.05$) due to NCD treatment. However, the group fed NCD supplemented with 0.5% bentonite showed larger liver than PCD but less than that of the NCD group.

Digestibility

Data presented in Table 4 revealed that increasing dietary bentonite supplementation to NCD diet was associated with a gradual increase ($P < 0.05$) in the digestion coefficient (DC) of dry matter (DM), ether extract (EE), ash, and crude fiber. The opposite was found in the NCD group; the DC of DM and CP significantly decreased ($P < 0.05$). There were no significant differences ($P > 0.05$) in DC of EE, ash, and CF between PCD and NCD.

Histopathological examination

The results showed that there were neither gross nor microscopic lesions in the liver, kidney, and intestine in rabbits fed positive control diet and NCD supplemented with 2% bentonite. While for the group fed NCD, the liver and kidneys were congested and the intestine was slightly healthy. The liver was enlarged and showed congestion of the portal vein, central vein, and hepatic sinosides with pressure atrophy of hepatic cells (Fig. 1). Some hepatocytes showed vacuolation and hydropic degeneration. The kidneys showed hydropic degeneration in some renal

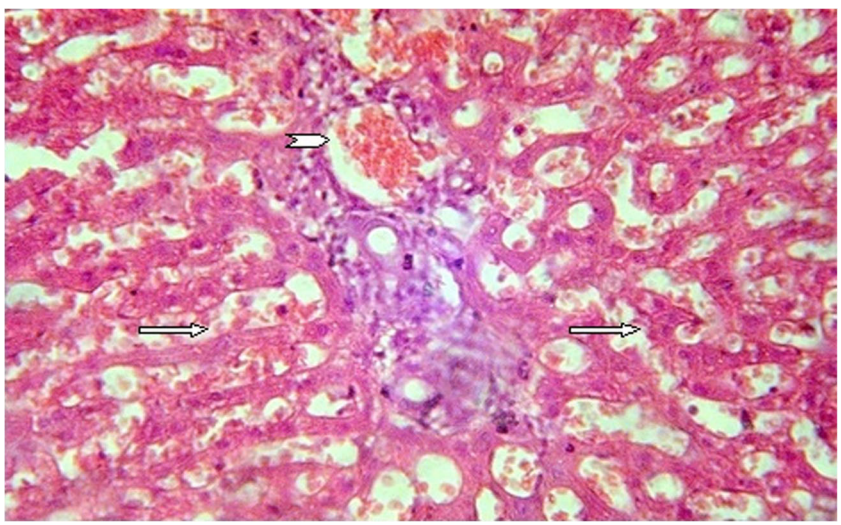

Fig. 5 The liver from the treatment NCD + 0.5% bentonite showing congestion of portal vein (arrow head) and hepatic sinosides (arrow) with pressure atrophy of hepatic cells, HE × 1200

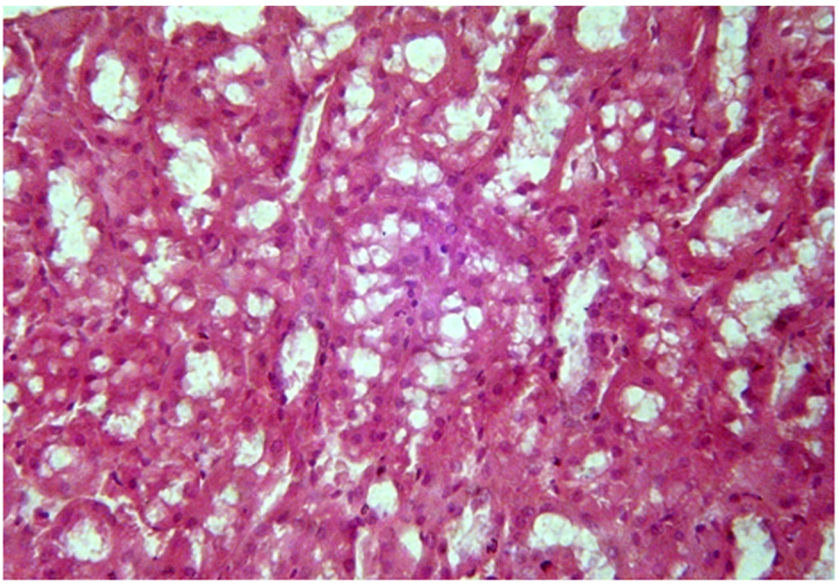

Fig. 6 The kidney from the treatment NCD + 0.5% bentonite showing hydropic degeneration in some renal epithelium, HE × 1200

epithelia (Fig. 2). The intestine showed catarrhal enteritis represented by desquamation in the lining epithelium of intestinal villi with hyperplasia and mucinous degeneration of the lining epithelium (Figs. 3 and 4) and hyperplasia of lymphoid follicle in submucosa. The results for the group fed NCD supplemented with 0.5% bentonite showed white foci in the cortex in the kidneys, and the intestine was normal. The liver showed hydropic degeneration in hepatocytes with perivascular aggregation of round cells (Fig. 5).The kidneys showed focal aggregation of round cells replaced necrotic renal tubules (Fig. 6). The lumen of some renal tubules showed cellular cast (Fig. 7).The intestine showed hyperplasia and mucinous degeneration of intestinal villi with edema in the submucosa (Fig. 8). The results for the group fed NCD supplemented with 1% bentonite showed that the liver, kidneys, and intestine were slightly normal.

Discussion

Several studies were carried out to explore new natural supplements to improve and enhance animal productivity. Among these supplements, aluminosilicates could be used for several purposes in the industry of rabbit. Bentonites are kind of aluminosilicates which exist in the market place due to their properties as mycotoxin adsorbents. In the current study, the efficacy of Egyptian bentonite in rabbit performance,

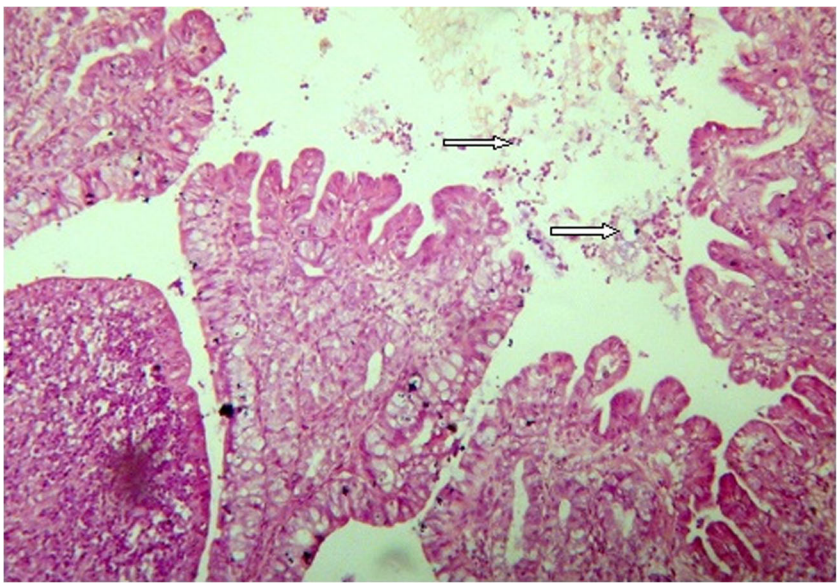

Fig. 7 The intestine from the treatment NCD + 0.5% bentonite showing catarrhal enteritis represented by desquamation in the lining epithelium of intestinal villi (arrow), HE × 300

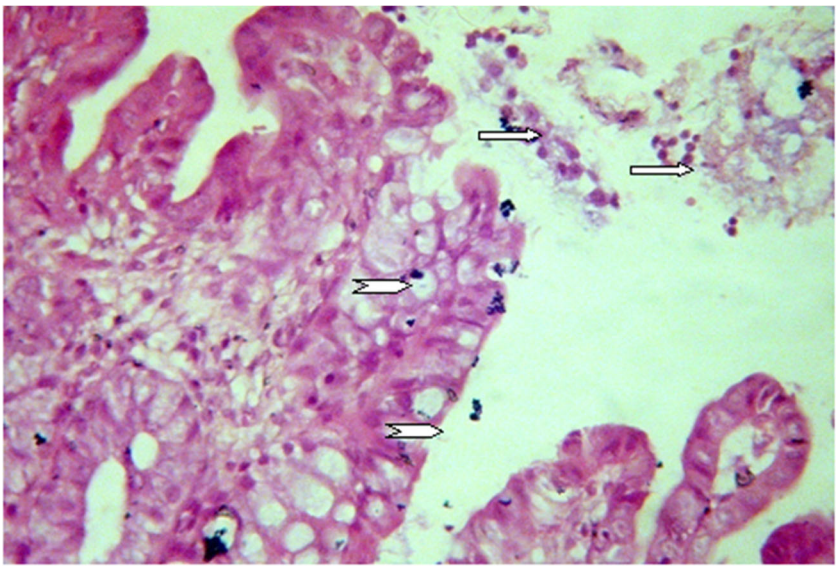

Fig. 8 High power of the intestine from the treatment NCD + 0.5% bentonite showing mucinous degeneration (arrow head) and desquamation in the lining epithelium of intestinal villi (arrow), HE × 1200

carcass traits, and digestibility in reducing the effects of AFB1 was assessed. Our results showed that bentonite supplementation was useful in reducing the negative effect of aflatoxin on the rabbit performance. These results could be due to improving the digestibility of the nutrients and reducing the negative effect of aflatoxin on animal health by reducing the histological lesions in the internal organs which were observed in aflatoxin-infected animals. By increasing the level of bentonite supplementation, the internal organs of the infected animals were similar to that of the control. This was also detected by the reduced relative weights of kidney and liver which were elevated in animals fed aflatoxin-contaminated diets. In line with our results, Pasha et al. (2008) demonstrated that birds fed diets enriched with sodium bentonite and treated with 0.5 or 1.0% acetic acid enhanced PER and digestibility of crude protein. This improvement may be due to the action of silicate minerals which enhance nutrients' digestibility. Silicates reduce the rate of passage through the gastrointestinal tract and consequently increase nutrient exposure to digestion. Similarly, Damiri et al. (2010) mentioned that bentonite swelling reduces feed rate of passage through the digestive tract and offers more time for effective feed utilization. The existence of montmorillonite in broilers' diet increased aminopeptidase, alkaline phosphatase, and maltase activities in the mucosa of small intestine (Ma and Guo 2008). Safaeikatouli et al. (2012) reported that the improvement in PER and ileal digestibility of protein was due to the presence of silicate mineral that prolonged feed passage time and improved nutrient metabolism. Shehata and Abd El-Shafi (2011) reported that Cu-BNT supplementation increased daily body weight gain and feed conversion; they attributed this improvement to the reduction of the total viable counts of pathogenic bacteria and increasing beneficial bacteria in the small intestine which reflected on improvement of the rate of passage, thickness of intestinal mucosa, nutrient digestibility, and absorption (Hu et al. 2002; Ye et al. 2003; Xia et al. 2005). Tatar et al. (2008) observed that zeolite supplementation increased the ileal digestibility of protein compared to control, attributing this to zeolite that can stimulate small intestine villas. Dos Anjos et al. (2015) showed that supplementing bentonite clay (BC) to the aflatoxin B1 (AFB1) diet reduced the severity of the histological lesions caused by aflatoxins and bentonite clay (0.5% diet) and the relative liver and kidney weights of chicks fed bentonite alone (0.5%) were similar ($P > 0.05$) to that of control chicks. Ramos et al. (1996) reported that removal of mycotoxins by different adsorbents added to mycotoxin-contaminated diets was thought to be effective in the gastrointestinal tract in a prophylactic rather than a therapeutic manner. Phillips et al. (1988) showed that HSCAS addition by 0.5% to chicken diets containing 7.5 mg/kg aflatoxin B1 resulted in a significant decrease in the inhibitory effects of aflatoxin on growth performance. They thought that the adsorption of HSCAS was chemisorptions including the formation of strong bonds. This binding mechanism was interpreted by Phillips et al. (1990) as the formation of a complex by the carbonyl system of the aflatoxin with "uncoordinated edge site" aluminum ions. Thus, HSCAS can act as a sponge sequestering aflatoxins in the gastrointestinal tract of farm animals. Neeff et al. (2013) reported that the addition of bentonite to the AFB1 diet significantly reduced the increase in relative weight of liver and kidney observed in chicks fed AFB1 alone. Rosa et al. (2001) have shown that the increase in relative weights of liver and kidney of chicks fed the AFB1diet could be reduced by bentonite addition (0.3%) to the AFB1 (5 mg/kg diet) diet. Lopes et al. (2006) demonstrated that supplementing bentonite to AFB1 diet improved BWG by 9.5%, but it was still lower than the performance of control birds. Abdl-Rahman et al. (2011) reported that supplementing

the rabbit diets with 2.5% bentonite with 1% urea led to improvement of the growth performance through improving ammonia utilization by cecal microbes.

Conclusion

Bentonite supplementation had a good role in preventing the negative effect of aflatoxin on rabbits' growth performance, decreased the relative weight of the liver and kidney which was increased by aflatoxin, caused significant improvement in the digestibility of the various nutrients, and decreased the histopathological lesions caused by aflatoxin-infected diets. The best level for ameliorating the aflatoxin effect was 2% bentonite. These results suggest that bentonite can be used to reduce the toxic effects of aflatoxin that may be present in rabbit feeds.

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Compliance with ethical standards The current study was conducted at the Rabbit Research Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. All the experimental procedures were carried out according to the Local Experimental Animal Care Committee and approved by the ethics of the institutional committee. Animals were cared for using husbandry guidelines derived from Zagazig University standard operating procedures.

Conflict of interest The authors have no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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