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The effect of sodium bentonite supplementation on growth, feed conversion rate and some biochemical parameters in rabbits fed on diet naturally contaminated with aflatoxins

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ABSTRACT:

Aflatoxins are metabolites generated by some molds, which can contaminate animal feed and cause serious health issues in humans and animals. Sodium bentonite, a type of clay, has been studied for its ability to ease the harmful effects of aflatoxins in animal diets. It is of great economic importance to find proper and easy methods to neutralize the harmful effects of aflatoxins that naturally occur in feed of animals. This protocol was designed to assess the effect of diet naturally contaminated with aflatoxins (B1, B2, G1 and G2) on the growth performance, feed conversion rate, some biochemical and antioxidant parameters, and the role of sodium bentonite to mitigate this harmful effect and also to recommend which dose is better to use (1 or 2.5 %). The results showed significant decrease in feed intake, weight gain, superoxide dismutase and reduced glutathione, this harmful effect was restored by sodium bentonite supplementation (especially with the higher dose 2.5%). The levels of alanine aminotransferase, aspartate aminotransferase, creatinine and urea in serum were elevated due to aflatoxin intake and their values were returned to normal by sodium bentonite supplementation (especially with the higher dose 2.5%).

INTRODUCTION:

Aflatoxins, the poisonous metabolites generated by *Aspergillus* species, pose a significant threat to animal health and food safety. Understanding the conditions that favor aflatoxin production is crucial for implementing effective control measures. Field contamination occurs when crops like corn and peanuts, which are susceptible to Aspergillus infection, are exposed to stressors such as drought or insect damage. These stressors weaken the plants' natural defenses, allowing molds to take hold and produce aflatoxins. Post-harvest, the risk continues as improper storage conditions,

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characterized by high humidity and warm temperatures, provide a breeding ground for mold proliferation. Additionally, insect damage to grains creates openings for mold spores to enter, exacerbating the problem **(Marai and Asker, 2008; Stathas et al. 2023; Kumar et al. 2021).**

It is essential to adhere to several practices to avoid aflatoxin contamination, including the use of resistant crop varieties, timely harvesting, proper storage conditions and adequate insect control. Cleaning and maintenance of equipment are necessary to avoid cross-contamination. By managing these factors, the incidence of aflatoxin contamination can be significantly reduced, protecting the well-being of animals and ensuring the safety of their products for human consumption **(Torres et al. 2014).**

A variety of approaches are used to detoxify aflatoxins including physical, chemical, and biological ones. Biological methods, such as the use of certain strains of bacteria or fungi which can biodegrade aflatoxins without leaving harmful residues. Chemical methods, while effective, must be carefully controlled to prevent the introduction of other toxic substances. Physical methods, including adsorption techniques, it envolves the binding properties of certain substances (i.e. sodium bentonite) to remove aflatoxins from contaminated products **(Vankayalapati, 2018).**

The consumption of aflatoxins-contaminated feedstuffs does not appear to harm animals directly, yet it does ultimately through other physiological systems **(Diekman and Green, 1992),** resulting in serious fertility issues, which is the most significant economic factor in the animal production trade **(James et al. 1992; El-Darawany and Marai, 1994).** Toxicity from aflatoxins-contaminated food includes carcinogenicity, hepatitis, nephritis, bile duct proliferation, liver fibrosis and cirrhosis, and genacologic forms **(Abd El Hamid and Dorra, 1993).**

Though challenges remain in terms of cost-effectiveness and maintaining the nutritional integrity of the feed. Ongoing research and development are crucial to improving these decontamination strategies, ensuring they are both safe for consumption and economically viable for producers. Therefore the present study aims to assess a neutral substance (sodium bentonite) with no side effect on animal or human health to bind with naturally occurring aflatoxins in feed and limit its harmful effect, and also to recommend the better dose to use whether it is 1% or 2.5%.

MATERIALS AND METHODS:

2.1. Ethical statement:

This study was approved by the institutional Animal Care and Use Committee (ARC-IACUC) (approval no. ARC/AHRI/130/24).

Materials:

Sodium bentonite: 100% powder was obtained from El sherook company for cosmetics.

Methods:

Six feed samples from different distributers in Cairo and Giza were collected and analyzed in feed deficiency unit, Nutritional deficiency diseases and Toxicology Department, Animal Health Research Institute, Giza. Amount of total aflatoxins (B1, B2, G1 and G2) in feed samples were determined using lateral flow test kit manufactured by ProGnosis Biotech S.A. according to **Gkanas et al. 2018**. The highest aflatoxins levels found in the obtained feed samples was 75 ppb, this sample was used in the present research.

The present experiment was conducted on 20 adult male New Zealand white rabbits. Basal diet and diet containing aflatoxin and/or sodium bentonite were provided throughout the time of the experiment daily (8 weeks). Sodium bentonite doses were chosen to compare between lowest and highest doses most commonly used in research. The first dose used was 1% sodium bentonite **(Shimaa et al. 2018)** and the higher dose was 2.5% **(Abdl-Rahman et al. 2011).** Animals used in the present study were classified into 4 groups as follows:

Group1: control negative (fed aflatoxin free diet and not treated with sodium bentonite)

Group 2: control positive (fed aflatoxin containing diet).

Group 3: Rabbits were fed aflatoxin containing diet and sodium bentonite was added to the feed (1 gm/100gm).

Group 4: Rabbits were fed aflatoxin containing diet and sodium bentonite was added to the feed (2.5 gm/100gm).

2.4. Growth performance parameters:

Over the entire period of the experiment (8 weeks) all rabbits were weighed weekly. The gain in the body weight was calculated as the difference between the initial and the final weight. Daily food intake was also recorded. The data obtained were used to calculate growth performance parameters (feed conversion).

2.5. Sampling:

After the end of the experiment blood samples were gathered from the rabbits by vein puncture. Blood samples were placed into clean, centrifuge tubes and centrifuged at 3000 rpm for 5 min. Sera were collected and stored at -20^{0}C to carry out biochemical assays. Serum samples were used to measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, reduced glutathione (GSH) and superoxide dismutase levels (SOD).

2.6. Biochemical parameters:

ALT and AST were determined by kits obtained from spectrum according to Breuer, 1996. Creatinine levels were determined by kits obtained from spectrum according to Bowers and Wong, 1980. Urea was determined by using kit obtained from Diamond Diagnostics according to Kaplan, 1984.

2.7. Antioxidant parameters:

Reduced glutathione was measured in serum by kits obtained from biodiagnistic according to the scheme described by Beutler *et al.,* 1963. Superoxide dismutase was measured in serum by kits obtained from biodiagnistic according to the technique described by **Nishikimi et al. 1972.**

2.8. Statistical analysis:

Analysis of data obtained from both growth parameters and biochemical investigations was performed using analysis of variance (ANOVA) processed by SPSS software version 20 (Statistical Package for Social Science), followed by "less significant difference" (LSD) with p>0.05 considered statistically significant.

RESULTS:

3.1. Growth performance parameters:

Feed intake and weight gain were significantly decreased by feeding the rabbits with aflatoxin containing diet, the decrease was partially restored to normal by sodium bentonite addition to the feed especially in the higher dose supplementation (2.5%). However, feed conversion rate didn’t show significant change from the control group (Table (1).

Biochemical parameters:

Serum levels of ALT, AST, creatinine and urea showed a significant elevation in the group fed with aflatoxins containing diet. Sodium bentonite ameliorated the harmful effect of aflatoxins getting all the biochemical parameters levels back to normal in the group given 2.5% sodium bentonite (Table (2).

Antioxidant parameters:

Aflatoxins caused significant decline in SOD and GSH levels in serum. GSH decreased levels were partially restored by sodium bentonite addition in feed (Table (3)).

Table 1. The effect of sodium bentonite (1 or 2.5 gm /100 gm feed) on growth performance parameters (feed intake, weight gain and feed conversion) of New Zealand white rabbits given diet containing aflatoxin during the experiment. Mean and standard error ($P<0.05$). Means with the same letter differ significantly

Growth performance parameters		Control	Aflatoxin containing diet	Aflatoxin containing diet and sodium bentonite (1 %)	Aflatoxin containing diet and sodium bentonite (2.5 %)
Feed intake (g)	Mean	$3604^{a,b}$	2437.75^{a}	2482.75^{b}	3014
	St. error	138	463.9	121.74	98.2
weight gain (final weight-initial weight)	Mean	$1450^{a,b}$	1087.5^{a}	967.5^{b}	1246.25
	St. error	61.98	55.4	77.39	55.65
Feed conversion (g feed/ g weight gain)	Mean	2.59	2.24	2.62	2.48
	St. error	0.19	0.14	0.17	0.2

Table 2. The effect of sodium bentonite (1 or 2.5 gm /100 gm feed) on biochemical parameters (ALT, AST, creatinine and urea) of New Zealand white rabbits given diet containing aflatoxin during the experiment. Mean and standard error ($P<0.05$). Means with the same letter differ significantly

Biochemical parameters		Control	Aflatoxin containing diet	Aflatoxin containing diet and sodium bentonite (1 %)	Aflatoxin containing diet and sodium bentonite (2.5 %)
ALT (U/L)	Mean	$31.45^{a,b}$	$47.34^{a,b,c}$	$40.77^{a,b,c}$	$30.27^{b,c}$
	St. error	0.75	0.63	0.35	0.76
AST (U/L)	Mean	36.84^{a}	$48.4^{a,b,c}$	38.54^{b}	35.88^{c}
	St. error	0.52	1.51	0.85	1.331
Creatinine (mmol/L)	Mean	$0.77^{a,\ b}$	$1.187^{a,b,c}$	$1.0175^{a,b,c}$	$0.83^{b,c}$
	St. error	0.01	0.024	0.017	0.024
Urea (mmol/L)	Mean	$19.565^{a,b}$	$42.35^{a,b}$	$37.77^{a,b,c}$	$21.09^{b,c}$
	St. error	0.549	0.57	0.56	0.65

Table 3. The effect of sodium bentonite (1 or 2.5 gm /100 gm feed) on antioxidant parameters (SOD, GSH) of New Zealand white rabbits given diet containing aflatoxin during the experiment. Mean and standard error ($P<0.05$). Means with the same letter differ significantly

Antioxidant parameter		Control	Aflatoxin containing diet	Aflatoxin containing diet and sodium bentonite (1 %)	Aflatoxin containing diet and sodium bentonite (2.5 %)
SOD (U/ml)	Mean	$7.43^{a,b,c}$	4.8^{a}	5.15^{b}	5.76^{c}
	St. error	0.46	0.3	0.2	0.23
GSH (mmol/ L)	Mean	17.16^{a}	14.49^{a}	15.9	15.75
	St. error	0.39	0.6	0.42	0.4

DISCUSSION

Aflatoxins are a specific assembly of mycotoxins secreted mostly by toxigenic Aspergillus species, particularly *A. flavus* and *A. parasiticus* that are well recognized contaminants in feed **(Jedziniak et al. 2019).** In 2020 according to the RASFF (Rapid Alert System for Food and Feed) database, aflatoxins presence was mainly detected in peanuts; spices; rice; and various nuts such as hazelnuts. Some of these foodstuffs have recently shown a high aflatoxin content exceeding 1000 µg/kg, this can lead to severe health problems, especially damage to the liver and other organs, primary liver cancer, and finally can lead to death **(Pickova et al. 2021a; Pickova et al. 2021b).** The presence of aflatoxins in feed is still a major dilemma to be solved.

Rabbits are known to be the most prone species to aflatoxin exposure **(Mézes, 2008). Hassan et al. (2016)** observed that aflatoxins produce a variety of symptoms in rabbit farms, such as anorexia, diarrhea, depression, weight loss, a decreased immunological response, and increased mortality.

It is of great magnitude to identify cost-effective and applicable methods to neutralize the harmful effects of aflatoxins that naturally occur in feed of animals. Several studies have been carried out on bentonite, an alumino-hydro-silicate material, that in one hand holds the ability to combine with many organic substances decreasing the bioavailability of aflatoxins and reducing its toxicity. On the other hand, it has non-nutritive value **(Mahendra et al. 2012).**

The present study was conducted to determine the effects of aflatoxin on the growth performance, feed conversion rate, some biochemical and antioxidant parameters, along with effects of sodium bentonite as a feed additive that can counteract this effect and to also to recommend which dose is better to use (1 or 2.5 %).

In the present study, the feed intake and body weight gain of rabbits fed aflatoxin containing ration decreased significantly. When aflatoxin-containing feed ration was treated with sodium bentonite (1 and 2.5%), the feed intake and growth performance of rabbits were improved significantly especially with the higher dose of bentonite. Our results agree with **Abu-EL-Zahab et al. 2012 and Nowar et al. 2001. Nowar et al. (2001)** recorded a significant decrease in average daily weight of rabbits fed diet naturally contaminated with 860 ppb aflatoxins that was restored by adding sodium bentonite with various concentrations to the feed (1, 2 or 3 %).

Studies have shown that aflatoxin-contaminated diets lead to decrease in feed intake and body weight gain in rabbits. This is primarily due to the harmful effects of aflatoxins on the liver and overall metabolism, causing anorexia and impaired nutrient absorption. Additionally, aflatoxins can disrupt the digestive system and lead to oxidative stress, further affecting growth and health. Feed intake may have been also low as a result of decreased palatability of aflatoxin contaminated ration **(Sorour et al. 2019; Mehta, 2013).**

The results showed significantly elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in response to consumption of aflatoxins-contaminated diet. These results are consistent with **Yousef et al. (2003) study on rabbits, and Abdel-Wahhab et al. (2006), and Abdel Wahab et al. (2002)** studies on rats.

Aflatoxins limit nitrogen and energy consumption from the ingested meal by negatively impacting the liver, which is considered the hub of human metabolism **(Reddy et al. 1991; Rotimi et al. 2019).** The serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been identified as early indicators of hepatocellular damage caused by aflatoxins exposure **(Abdel-Wahhab and Aly, 2003)**.

The buildup of aflatoxins in the liver may cause cell death and inflammation. During aflatoxicosis, AFB1 is primarily metabolized in the liver, its reactive metabolite can attach to cellular macromolecules such as proteins, lipids, and nucleic acids, resulting in apoptosis of liver cells and ultimately liver damage **(Ismail et al. 2020).** When hepatocyte permeability is elevated after hepatic damage, aminotransferases might be discharged into the circulation, increasing serum ALT and AST levels **(Rashidi et al. 2020).**

In our study, serum levels of urea and creatinine of the aflatoxin fed rabbits significantly increased compared with the control group. Creatinine levels were significantly elevated in agreement with **Nashwa and Atiyah (2020) and Verma and Raval (1997)** findings in rabbits and rats ingested aflatoxin. In agreement with our study, **Hassan et al. 2019** recorded that feeding aflatoxin-containing diet to rabbits reduced their weight gain, average daily gain and feed conversion ratio. Also, the serum samples taken from rabbits fed with aflatoxin-contaminated diet showed increased concentrations of urea, creatinine, ALT and AST. Adding sodium bentonite at a dose of 5 g/kg diet reduced the damaging effects of aflatoxin B1 on growth performance and biochemical parameters.

This significant increase in kidney function tests (urea and creatinine) may be due to decreased excretion from the kidney reflecting compromised kidney functions and glomerular filtration as a result of AF toxicity which agreed with **Orsia et al. (2007), Abdou et al. (2015) and Ismail et al. (2020).**

SOD and GSH levels decreased significantly in rabbits fed on aflatoxin-containing diet compared to the control group. Similarly, **Choudhary and Verma (2005) studies in mice, Rastogi et al. (2001) in rats and Dönmez and Keskin (2008)** in rabbits found decreased SOD and GSH levels inflicted by aflatoxicosis. The metabolites of aflatoxins cause cellular oxidative stress by enhancing lipid peroxidation processes, potentially upsetting the antioxidant/oxidant system balance **(Muhammad et al. 2018).**

Alharthi et al. 2022 recorded that broiler treated with 0.25 mg/kg AFB1 showed reduced daily weight gain during grower, finisher, and overall periods, decreased serum concentrations of total antioxidant capacity (T-AOC), and total superoxide dismutase (T-SOD) and raised serum activities of ALT and AST. Feeding 0.4% bentonite to aflatoxin contaminated diet increased daily weight gain, and serum levels of T-AOC, and T-SOD and decreased serum ALT and AST activities.

CONCLUSION

In conclusion, introducing sodium bentonite into rabbit diets (especially the higher dose of 2.5%) can be cost-effective, safe, and practical technique to prevent aflatoxicosis and improve overall health and performance in rabbits. Therefore, it is recommended to use sodium bentonite as a supplement with concentration of 2.5% to avoid aflatoxicosis.

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