EFFECT OF CARBON MATERIAL AND BENTONITE ON REDUCTION THE NEGATIVE IMPACTS OF DEOXYNIVALENOL IN RAT ORGANISM

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ABSTRACT

The aim of this study was to investigate the efficacy of mycotoxin adsorbents based on graphene oxide (GO), purified bentonites, and their combination in reducing the negative impact of deoxynivalenol (DON). The experiment involved 48 rats (190 ± 5 g), divided into 8 groups of 6 each. Groups one to seven received a diet containing a high level of DON. Groups one to six had their feed treated with mycotoxin adsorbent. Group one (T1) received a diet with GO (0.25 g/kg), group two (T2) a diet with GO (0.5 g/kg), group three (T3) a diet with purified bentonite (1 g/kg), group four (T4) a diet with purified bentonite (2 g/kg), group five (T5) a diet with GO (0.25 g/kg) and purified bentonite (1.75 g/kg), and group six (T6) a diet with GO

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(0.5 g/kg) and purified bentonite (1.5 g/kg). The control group (C) was fed a diet without DON. The Cmyko group received a diet with mycotoxins without adsorbent. Evaluated parameters included weight and liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). No statistically significant difference in body weight was observed between groups C and T1–T6 or between groups Cmyko and T1–T6. For liver enzymes, no statistically significant differences were observed in ALP. ALT activity was higher in groups T1, T4, T5, T6, and Cmyko compared to group C. AST activity showed statistically significant differences in all test groups (T1–T6) compared to groups Cmyko and C. In conclusion, a higher dose of GO (0.5 g/kg), purified bentonite, and their combination may be beneficial in mitigating the toxicity of DON.

Keywords: mycotoxins; bentonite; graphene oxide

INTRODUCTION

Mycotoxin contamination in feed is a serious global issue, affecting up to 80% of crop production (Kos et al., 2024). Over 900 mycotoxins have been identified (Habauzit, Lemée, and Fessard, 2024) Their widespread presence in feed, combined with their known adverse effects on livestock health, makes them a significant safety and economic concern (Palumbo et al., 2020).

Adsorption is a surface phenomenon where mycotoxins bind to materials through electrostatic and polar interactions (Freire, Ali, and de Oliveira, 2022). Bentonite, a phyllosilicate clay with a layered crystalline structure, shows variable adsorption efficiency depending on the content of montmorillonite and exchangeable cations (Vila-Donat et al., 2018). Adsorption mechanisms include chemosorption, electron exchange, hydrogen bonding, ionic interactions, and coordination with carbonyl groups, similar to nutrient adsorption in animal diets (Elliott, Connolly, and Kolawole, 2020). Montmorillonite effectively binds polar mycotoxins such as aflatoxins (AFL), but its efficacy against deoxynivalenol (DON) is limited, despite DON's reactive features such as an epoxide ring, hydroxyl groups, and an α,β-unsaturated carbonyl group (Horky et al., 2021).

Graphene oxide (GO) is a promising material for DON adsorption, however, it was also confirmed that GO has non-specific binding properties for micronutrients (Horky et al., 2020). Its surface, containing oxygen functional groups, contributes to its hydrophilicity (Bytesnikova, Adam, and Richtera, 2021). The unique molecular structure of GO allows it to interact with target molecules through charge transfer, π-π interactions, and hydrogen bonding (Ji and Xie, 2020; Zhou et al., 2023).

The study aimed to assess the impact of purified bentonite and GO on growth performance and liver parameters in rats. We used purified bentonite, which has improved adsorption capacity due to increased binding sites, and GO, known for its effective binding to DON.

MATERIAL AND METHODS

The experiment was conducted at the experimental facility of the Department of Animal Nutrition and Forage Production at Mendel University in Brno, in accordance with the Animal Protection Act No. 246/1992 Coll. The experiment was approved by the Ministry of Education, Youth, and Sports under reference number MSMT-15228/2019-4.

Microclimatic conditions in the laboratory were monitored, primarily limited by temperature, which was measured using a "DATALOGER S 3120 (Voltcraft, Germany)" and maintained within the range of 23 °C ± 1 °C. Additionally, constant air humidity was monitored with the same device and maintained at 60% using an air conditioning unit. The photoperiod was artificially controlled according to a 12-hour light and 12-hour dark cycle with a maximum intensity of 200 lx.

Male Wistar albino rats of the outbred strain were used as the experimental model for this study. The average weight of the rats at the beginning of the experiment was 190 ± 5 grams. The experimental observation lasted for 28 days. The rats were housed in plastic boxes with grates. Throughout the duration of the experiment, the experimental animals had ad libitum access to water and feed. The animals were weighed weekly. Daily monitoring included feed intake and health status. The rats were divided into 8 groups, with each group consisting of 6 males. The first to seventh groups of animals were fed a diet with a high content of DON (4430 µg/kg diet). The first group (T1) of animals had GO added to their feed at a dose of 0.25 g/kg diet. The second group (T2) received GO at a dose of 0.5 g/kg diet. The third group (T3) of rats had purified bentonite mixed into their diet at a dose of 1 g/kg diet. The fourth group (T4) received purified bentonite at a dose of 2 g/kg diet. The fifth group (T5) received a combination of GO at a dose of 0.25 g/kg diet + purified bentonite 1.75 g/kg diet. The sixth group (T6) had a combination of GO at a dose of 0.5 g/kg diet + purified bentonite 1.5 g/kg diet mixed into their diet. The seventh group (Cmyko) was fed a DON diet with defined mycotoxin content without the addition of adsorbents. The eighth control group (C) of rats was fed a diet with mycotoxin content below the harmful threshold (DON – 238 µg/kg diet).

Table 1. Composition of the rat feed mixture (%)

Component	(%)
Maize *	42.9
Wheat *	38.6
Soybean meal extracted (48%)	15.4
Lysine	1.3
Mineral-vitamin premix	1.8

******In the experimental groups of rats, corn and wheat were the main sources of DON, while the control group did not have above-limit values of DON in these two ingredients.*

The weight of the animals was monitored at regular weekly intervals. After 28 days of the experiment, the rats were euthanized (2 hours after the last feeding). Blood was collected 28 days after anesthesia through cardiac puncture. Blood samples were analyzed for biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using the ELISA method as described by Horky et al. (2021).

Data were processed using the statistical software R. The effect of adsorbents on the live weight of rats was tested using one-way analysis of variance. For liver enzyme values, a generalized linear model (GLM) with a gamma distribution was employed. Post hoc analysis compared the treatment groups to the control (C) and Cmyko using Dunnett's test. The null hypothesis was rejected at $p < 0.05$.

RESULTS AND DISCUSSION

Determining the body weight of rats was used to evaluate the performance of animals (Figure 1). Administration of DON at a dose of 4430 μg/kg can negatively impact growth performance in rats, which is also confirmed by the results of Holanda and Kim (2020) at a dose of 3.2 mg/kg DON (Holanda and Kim, 2020). The presence of DON in the feed may lead to feed rejection, consequently decreasing weight gain (Xu et al., 2022). Several studies have demonstrated reduced nutrient digestibility in poultry and pigs in the presence of DON, leading to impaired growth due to histopathological changes in the intestine (Liu et al., 2020; Gallo et al., 2020). Award et al. (2011) found that DON inhibits jejunal villus growth, reducing nutrient absorption (Awad et al., 2011). In our research, no statistically significant differences in the efficacy of the adsorbents among the groups T1–T6 compared to C and Cmyko were observed, however the Cmyko group showed reduced weight gain.

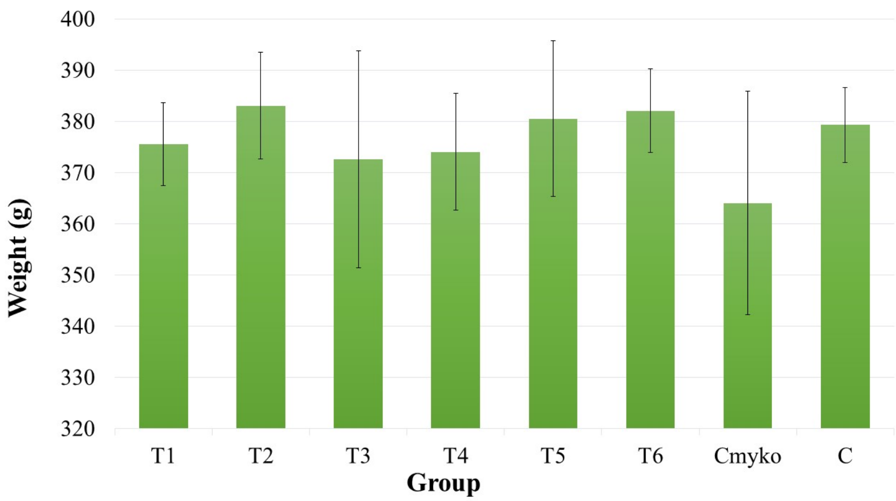

Figure 1: Live weight according to groups

Adsorbents mitigate the harmful effects of mycotoxins by binding to them, reducing their bioavailability and intestinal absorption (Xu et al., 2022). Bentonite's adsorption effects are linked to the physical and chemical properties of mycotoxins (Adegbeye et al., 2020). Horky et al. (2021) reported no significant changes in pig growth with purified bentonites, their study showed improved growth of animals at a dose of 1.5 kg/t (Horky et al., 2021; Horky et al., 2022). Ghazalah et al. (2021) found that bentonite enhanced broiler performance and health. Carbon-based materials vary in their ability to adsorb DON based on type and activation proces (Ghazalah et al., 2021). Carbon-based materials vary in their ability to adsorb DON depending on the type and activation proces (Vila-Donat et al., 2019). Horky et al. (2020) reported that GO adsorbs DON at 1.69 mg/g, with optimal results at 37 °C and pH 5 (Horky et al., 2020). Groups T2, T5, and T6 showed the better growth compared to C and Cmyko, suggesting GO's strong binding capacity for DON. Soybean residue-derived carbon materials achieved up to 98.82% DON adsorption efficiency (Ying et al., 2021) and and Pirouz et al. (2017) demonstrated a 69.6% reduction in DON levels using magnetic graphene oxide (Pirouz et al., 2017).

The liver is essential for metabolism, digestion, detoxification, and excretion. Key liver function tests include ALP, ALT, and AST. Elevated levels of these enzymes can indicate hepatocellular damage, as liver injury causes the release of enzymes from damaged hepatocytes, resulting in increased serum AST and ALT (Martinez et al., 2023; Amer et al., 2022). AST is found in the liver, heart, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and red blood cells, and its elevation can be due to non-hepatic factors. In contrast, ALT is liver-specific, and its increase directly suggests hepatocellular damage

(Kalas et al., 2021). Biochemical assessments should consider the age and sex of rats, as AST, ALT, and ALP levels are higher in males and decrease with age (Patel et al., 2024).

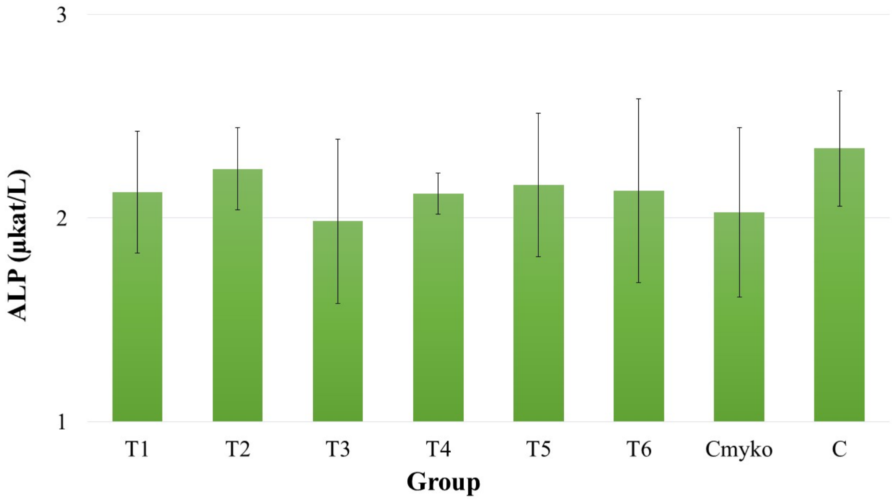

Figure 2: Level of the enzyme ALP (µkat/L)

Enzyme activities in our study were compared across the control group (C), the mycotoxicosis group (Cmyko), and the treated groups (T1–T6) (Figures 2–4). No statistically significant differences were found in ALP values (Figure 2, A).

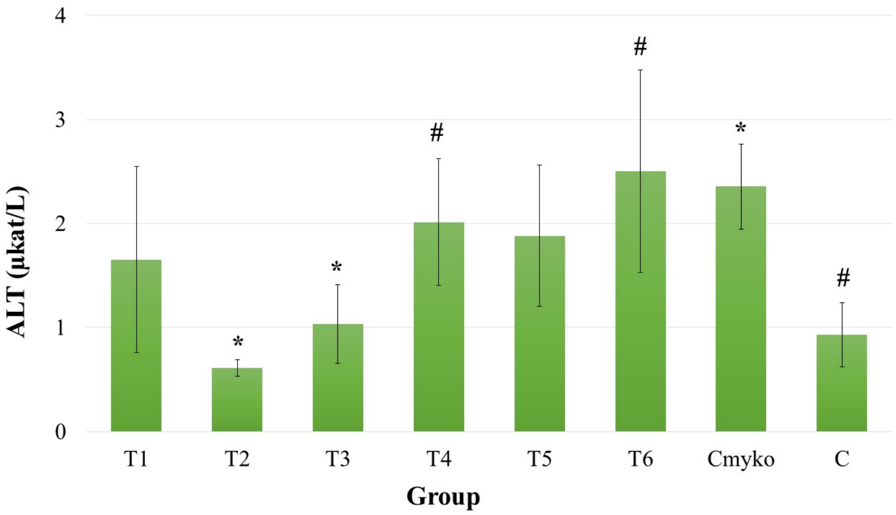

Figure 3: Level of the enzyme ALT (µkat/L)

******The group marked with this symbol is statistically significant compared to the group Cmyko; # the group marked with this symbol is statistically significant compared to the group C*

Significant differences in ALT levels were observed in groups T2, T3, and T6 compared to the mycotoxicosis group (Cmyko) and the control group (C) (Figure 2, B). ALT levels were elevated in groups T4 to T6. Patel et al. (2024) report that normal ALT levels in rats range from 0.5 to 1 µkat/L. (Patel et al., 2024). Slight increases in ALT were noted in groups T1 and Cmyko. ALT activity was higher in groups T1, T4, T5, T6, and Cmyko compared to C, suggesting that purified bentonite and the bentonite + GO combination may negatively impact liver function by increasing ALT levels compared to C.

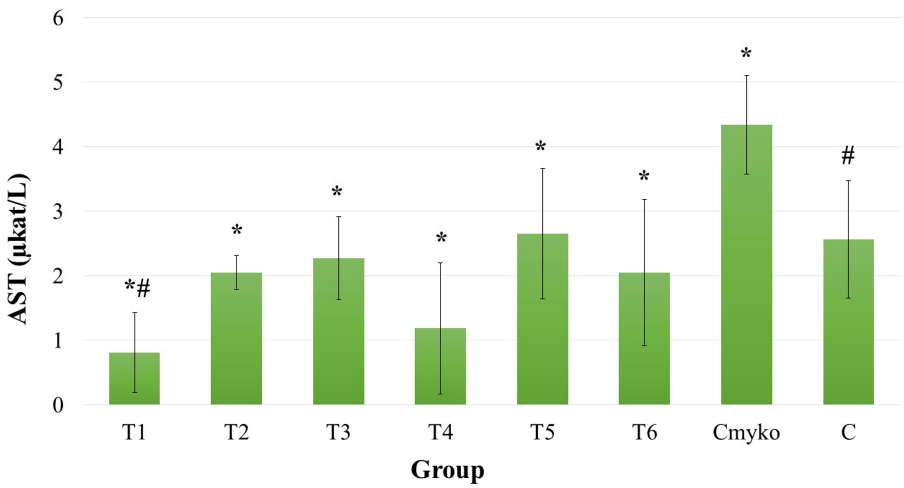

Figure 4: Level of the enzyme AST (µkat/L)

**The group marked with this symbol is statistically significant compared to the group Cmyko; # the group marked with this symbol is statistically significant compared to the group C*

Statistically significant differences in AST values were observed in all groups (T1–T6) compared to the Cmyko and the C (Figure 2, C). Significantly lower AST levels were observed in group T1 compared to Cmyko and C. Additionally, significantly lower AST levels were observed in groups T2 through T6 compared to Cmyko. According to Patel et al. (2024), the reference range for AST in rats is 1.4 – 3.78 µkat/L. (Patel et al., 2024). Elevated levels of AST and ALT may indicate hepatic mycotoxicosis (Mgbeahuruike et al., 2018). Increased serum levels of these enzymes were also found in rabbits fed AFL-containing diets (Hassan et al., 2019) and similar results were reported in other mycotoxin studies (Yaman, Yener, and Celik, 2016; Ejiofor et al., 2021). AST levels were lower in groups T1 and T4 compared to the control group (C), while groups T2, T3, T5, and T6 remained within the reference range.

High-dose GO (0.5 g/kg) significantly reduced ALT activity compared to Cmyko, while low-dose GO (0.25 g/kg) significantly reduced AST levels compared to Cmyko and C.These results suggest that higher doses of GO may offer better liver protection against mycotoxins, which is consistent with previous studies (Fu et al., 2015). The T3 group, with a lower dose of purified bentonite, showed similar AST and ALT trends as the higher dose in T2. The T4 group, receiving a higher dose of purified bentonite, exhibited a trend similar to T1. The results indicated that graphene oxide (0.5 g/kg) provided the best protective effect, followed by purified bentonite (1 g/kg), the combination of GO (0.25 g/kg) and purified bentonite (1.75 g/kg), and finally the combination of GO (0.5 g/kg) and purified bentonite (1.5 g/kg).

CONCLUSION

This study aimed to evaluate the impact of two adsorbents at various doses and combinations on rat weight and liver enzymes following exposure to deoxynivalenol (DON). Our research did not reveal significant changes in the growth performance of rats; however, trends were observed in liver enzymes, specifically ALT and AST, compared to the control and mycotoxin-contaminated diet. GO at a dose of 0.5 g/kg demonstrated the most effective protective action against DON-induced mycotoxicosis, followed by purified bentonite at 1 g/kg, and combinations of GO (0.25 g/kg) with purified bentonite (1.75 g/kg) and GO (0.5 g/kg) with purified bentonite (1.5 g/kg). Overall, neither purified bentonite, GO, nor their combinations adversely affected the health status of the rats.

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