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EFFECTIVENESS OF COMPOSITE FEED ADSORBENTS IN BINDING MAJOR MYCOTOXINS UNDER VARIABLE PH CONDITIONS

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ABSTRACT

Mycotoxins are toxic secondary metabolites produced by fungi of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps* and *Alternaria* and represent a significant threat to the safety of cereal crops and animal feed. These compounds may accumulate at various stages of production, storage, and transportation, which necessitates the development and application of effective strategies to reduce their toxic effects. The aim of the study was to perform a comparative evaluation of the adsorption capacity of three mycotoxin-binding agents. The study was conducted under in vitro conditions; therefore, the adsorption efficiency observed may differ under in vivo gastrointestinal conditions. Three commercial mycotoxin binders were tested, each containing 70% inorganic sorbent from the silicate group (bentonite, zeolite, or diatomite) and 30% organic component based on yeast cell walls (sources of mannan oligosaccharides and β-glucans). In preparation No. 1, the organic fraction consisted of yeast cell walls containing 33% MOS and 25% β-glucans; preparation No. 2 contained 20% MOS and 35% β-glucans; and No. 3 contained 20% MOS and 20% β-glucans. The adsorption capacity of the mycotoxin-binding preparations was determined under laboratory conditions using the adsorption isotherm method. All experiments were conducted in eight replicates. The results showed that the highest adsorption efficiency of deoxynivalenol (DON), aflatoxin B1, and zearalenone (ZEA) was observed under acidic conditions (pH 3). The highest sorption of DON, ZEA, and fumonisin B1 (FB1) was achieved by sorbent No. 1 (containing 33% MOS and 25% β-glucans), especially at pH 3. In addition, sorbent No. 1 demonstrated 100% binding of aflatoxin B1 across the entire pH range studied (pH 3, 5, and 7), indicating the stability of its adsorption properties. In contrast, sorbents No. 2 and 3 exhibited relatively low and unstable activity against fumonisin B1; at pH 7, the values ranged from 0.4% to 2.8% The results obtained indicate that sorbent No. 1 can be considered the most versatile and effective for binding the major mycotoxins evaluated in this study.

Keywords: *Fusarium*, *Aspergillus*, aflatoxin B1, deoxynivalenol (DON), zearalenone (ZEA), fumonisin B1 (FB1), mycotoxin binders

INTRODUCTION

The quality and safety of raw materials are critical for the production of safe feed, directly affecting animal health, the safety of animal-derived products for human consumption, and environmental sustainability. To ensure safety throughout the agri-food chain, compound feed manufacturers and accredited laboratories routinely monitor raw materials and finished products for contamination, conducting daily analyses of selected samples **(Park, 2018; Kim, 2023; Perera & Ravindran, 2025).**

The risk associated with the emergence of novel and combined toxic agents in the agri-food chain is continuously increasing. Among priority contaminants, mycotoxins-secondary metabolites of microscopic fungi-occupy a leading position and have been recognized as one of the most significant threats to biosafety in recent decades (**Deng *et al.*, 2023**). To date, more than 400 mycotoxins have been identified; however, the greatest threat to animal productivity and immune function is posed by aflatoxins (AF), fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin, and ochratoxins (OTA) (**Bryden, 2012; Mijić *et al.*, 2024**).

The primary route of mycotoxin entry into the animal organism is the gastrointestinal tract. Absorption may begin in the oral cavity and stomach, reaching its maximum in the small intestine. Following entry into the portal circulation, mycotoxins undergo biphasic biotransformation in the liver, including phase I reactions (oxidation, reduction, and hydrolysis) followed by phase II conjugation. These processes enhance the polarity of toxin molecules, thereby facilitating their excretion (**Liew *et al.*, 2018; Murtaza *et al.*, 2024**).

The mitigation of mycotoxin toxicity has been extensively addressed in the scientific literature (**Huss *et al.*, 2018; Das *et al.*, 2023; Quesada-Vázquez *et al.*, 2024**). Contemporary strategies for reducing their adverse effects include physical approaches (thermal treatment, irradiation, cold plasma, and adsorption), chemical methods (oxidizing and reducing agents, plant extracts, essential oils, and phytochemicals), and biological strategies (microbial degradation, microbial antagonism, and enzymatic detoxification) (**Agriopoulou *et al.*, 2020; Yao *et al.*, 2026**).

The efficacy of modern enterosorbents is determined by their capacity to irreversibly bind toxins over a broad pH range, their high selectivity (i.e., minimal interaction with vitamins and trace elements), and their economic feasibility in animal nutrition (**Kihal *et al.*, 2022**). The adsorption capacity of these agents is largely governed by the molecular structure, polarity, and degree of ionization of mycotoxins, which in turn dictate the nature of physicochemical interactions between toxin molecules and the sorbent surface (**Deng *et al.*, 2010; Kihal *et al.*, 2020; Greco *et al.*, 2026**).

The mechanism of action of binders such as clay minerals, activated carbon, and yeast cell wall components is based on a combination of chemical and physical interactions. Chemical interactions include cation exchange, ion-dipole interactions, Van der Waals forces, and hydrogen bonding, whereas physical factors involve pore size as well as the spatial configuration and geometry of mycotoxin molecules (**Kihal *et al.*, 2022**).

Currently, both inorganic and organic adsorbents are widely used in animal production. Mineral components (e.g., bentonite, zeolite, and aluminosilicates) bind toxins primarily through ion-exchange processes and surface adsorption. In contrast, organic sorbents derived from *Saccharomyces cerevisiae* cell walls interact with mycotoxins via hydrogen bonding, hydrophobic interactions, and electrostatic forces (**Luo *et al.*, 2020; Liu *et al.*, 2021**).

Recent studies highlight the advantages of composite formulations that combine a mineral matrix with yeast-derived polysaccharides such as β-glucans and mannan-oligosaccharides. These systems provide a broader detoxification spectrum and improved stability across varying pH conditions (**Vila-Donat *et al.*, 2018; Kihal *et al.*, 2023**). Notably, the combination of sepiolite with organic polymers such as calcium lignosulfonate (at an 80:20 ratio) has been shown to significantly enhance the sequestration of DON, OTA, and ZEA (62–82%) under gastrointestinal conditions (**Żybura & Jedziniak, 2024**).

It is important to recognize that adsorption is a dynamic process that varies throughout passage along the gastrointestinal tract. Recent work by Greco *et al.* (2022), evaluating a novel hybrid binder composed of trioctahedral smectite and a lignocellulose-based material, demonstrated distinct pH-dependent patterns. The adsorption of AFB_1, ZEA, and T-2 was independent of pH within the range of 3–9, whereas OTA and FB_1 adsorption occurred primarily at pH 3–5. Notably, AFB_1, ZEA, and T-2 remained stably bound upon increasing pH from 3 to 7, while desorption of FB_1 and OTA did not exceed 38% (**Greco *et al.*, 2022**).

In parallel with physicochemical sorption, biological detoxification strategies are actively being developed. Studies by **Adunphatcharaphon *et al.* (2021)** and **Jard *et al.* (2009)** demonstrate the feasibility of ZEA detoxification using *Lactobacillus plantarum* BCC 47723 and conidia of *Aspergillus japonicus*. Similarly, Zhou *et al.* (2017) proposed the removal of T-2 toxin using the extracellular fraction of *Lactococcus lactis* CAMT22361.

A notable example of biological detoxification is the strain *Planococcus* sp. S118, which exhibits a high capacity for zearalenone (ZEA) removal via physical binding. Heat-inactivated cells showed significantly higher adsorption efficiency (47.82%) compared to viable cells (21.82%), suggesting the exposure of additional binding sites on the cell surface following thermal, acid, or Triton X-100 treatment. The efficiency of this process was strongly influenced by incubation conditions, including bacterial concentration ($\geq 5 \times 10^7$ CFU/mL), temperature (30 °C), and acidic pH (4.5), corresponding to gastric conditions where initial toxin binding occurs (**Lu, *et al.*, 2011**).

Beyond physical adsorption, microbial detoxification may also proceed via biotransformation, providing an irreversible effect through modification of toxicophore groups within the toxin molecule. For example, the laccase CotA from *Bacillus licheniformis* can efficiently transform aflatoxin B_1 (AFB_1) into less toxic metabolites such as AFB_1-Q_1 by targeting the furan and lactone rings. Molecular docking studies have shown that hydrogen bonding and Van der Waals interactions play a crucial role in stabilizing the AFB_1–CotA complex (**Sun *et al.*, 2023; Guo *et al.*, 2025**).

The integration of such microbial agents with optimized mineral sorbents, as demonstrated in the models of Feizy *et al.* (2025), represents a promising advancement in the development of broad-spectrum protective systems. Given the high resilience of mycotoxins produced by fungi of the genera *Fusarium, Aspergillus, Penicillium, Claviceps,* and *Alternaria*, current research is focused on two main directions: the development of innovative binders with enhanced affinity and the identification of compounds that support intestinal barrier function (**Vila-Donat *et al.*, 2018; Deng *et al.*, 2023**).

Based on the above, the aim of this study was to investigate and comparatively evaluate sorption and biodegradation approaches for mycotoxins produced by micromycetes of the genera *Fusarium* and *Aspergillus* using complex adsorption systems.

MATERIALS AND METHODS

To evaluate sorption capacity, three commercial mycotoxin-binding agents were tested. Each product contained 70% inorganic sorbent from the silicate group (bentonite, zeolite, or diatomite) and 30% organic component based on yeast cell walls (sources of MOS and β-glucans). Preparation No. 1 contained 33% MOS and 25% β-glucans; preparation No. 2 contained 20% MOS and 35% β-glucans; and preparation No. 3 contained 20% MOS and 20% β-glucans. The in vitro binding efficiency of the sorbents was evaluated in reaction mixtures at pH 3, 5, and 7 and at a temperature of 37 °C for 2 hours. The selection of pH values was intended to simulate different sections of the animal gastrointestinal tract. Specifically, pH 3 corresponds to the acidic conditions of the stomach, pH 5 simulates the transitional environment of the proximal small intestine, and pH 7 corresponds to the neutral or slightly alkaline conditions of the distal small intestine. This experimental design made it possible to assess the stability of the sorption capacity of the studied preparations under various physiological conditions of the digestive tract. The sorption capacity of the mycotoxin-binding preparations was determined under laboratory conditions using the adsorption isotherm method. Toxin solutions with different initial concentrations were mixed with a fixed amount of sorbent. Each experimental sample contained the sorbent, a buffer solution adjusted to the required pH, and a standard solution of the corresponding mycotoxin. For sample preparation at pH 3, 5, and 7, 100 mg of sorbent was incubated with 5 mL of phosphate–saline buffer (4 g sodium chloride, 1.8 g disodium hydrogen phosphate, 0.1 g potassium chloride dissolved in 500 mL of distilled water; phosphoric acid was added to adjust the pH) for 2 hours at 37 °C. After incubation, the samples were centrifuged at 3,500 rpm for 15 minutes. Subsequently, 4.5 mL of the supernatant was transferred to a test tube, diluted with 10 mL of distilled water, and analyzed using liquid chromatography–tandem mass spectrometry (LC–MS/MS). The amount of toxin bound by the sorbent was calculated as the difference between the initial toxin concentration and the residual concentration in the supernatant. The results obtained at different initial toxin concentrations were fitted to adsorption isotherm models. Based on these data, the main adsorption parameters were determined, in particular the maximum sorption capacity (qmax). Each treatment was prepared in eight independent replicates (n = 8), and each extract was analyzed in two technical replicates.

Statistical analyses

Statistical analysis was conducted in R (version 4.5.2) using Tukey's HSD test implemented in the agricolae package. For each mycotoxin, the arithmetic mean ($\bar{X}$) and standard deviation (SD) were calculated. Differences among groups were considered statistically significant at $P < 0.05$. Boxplots (indicating the median, percentiles, and outliers) were generated using MedCalc for Windows, version 20.2 (MedCalc Software, Ostend, Belgium, 2022).

RESULTS AND DISCUSSION

Modern industrial animal production requires strict cost optimization, with feed accounting for up to 70% of total production costs. Ensuring feed safety through the use of adsorbents represents a strategic approach to protecting animal health. Although *in vivo* studies remain the "gold standard," their high cost underscores the critical role of *in vitro* models that simulate gastrointestinal tract (GIT) conditions for the rapid screening of effective products (**Boudergue *et al.*, 2009; Greco *et al.*, 2026**).

The process of mycotoxin binding is multifactorial and is based on non-covalent interactions, including hydrophobic interactions, hydrogen bonding, and Van der Waals forces. The efficiency of these interactions is strongly influenced by environmental pH and the physicochemical properties of the toxins, such as polarity and molecular size (**Greco *et al.*, 2019**).

It has been demonstrated that an effective protective strategy should rely on the use of multi-component adsorbents capable of simultaneously binding different chemical groups of toxins, thereby preventing their synergistic effects (**Holanda & Kim, 2021**). In the present study, three mycotoxin-binding formulations were evaluated, each containing 70% inorganic sorbent (bentonite, zeolite, or diatomite) and 30% organic component in the form of yeast cell walls. The selection of yeast cell walls as the organic fraction is justified by their high biological activity: mannan-oligosaccharides (MOS) and β-glucans are known to modulate gut microbiota, stimulate immune responses, and interact with toxin molecules via hydrogen bonding, hydrophobic interactions, and electrostatic forces (**Teng *et al.*, 2017; Spring *et al.*, 2000; Teng & Kim, 2021;**).

Our results confirmed that the adsorption capacity of the tested formulations was significantly dependent on pH ($P < 0.05$), which is consistent with the findings of **Kihal *et al.* (2022)** regarding the pH-dependent adsorption behavior of yeast cell wall components (YCW).

Among the tested formulations, sorbent No. 1 (containing 33% MOS and 25% β-glucans) demonstrated the highest adsorption capacity for all investigated mycotoxins. Specifically, the maximum adsorption of deoxynivalenol (DON) was observed at pH 3 (65.3 ± 6.9%), with a gradual decline in efficiency as pH increased (down to 52.7 ± 6.4% at pH 7). This finding is particularly important, as most commercial binders—except activated carbon-are reported to exhibit limited capacity for DON sequestration (**Ahn *et al.*, 2022**).

The adsorption of fumonisin B_1 (FB_1) by sorbent No. 1 was moderate and strongly pH-dependent: the highest values (25.9 ± 1.6%) were observed at pH 3, whereas efficiency decreased markedly under neutral conditions. This is consistent with the findings of **Greco *et al.* (2022)**, who demonstrated that fumonisin binding is most effective in acidic environments (pH 3–5), likely due to changes in the ionization state of the toxin molecules. In contrast, the adsorption of zearalenone (ZEA) remained relatively stable (≈47%) under both acidic and neutral conditions, with only a slight decrease at pH 5 (Table 1).

Table 1 Comparison of the sorption capacity of three sorbents toward mycotoxins at different pH values pH (x ± SD, n = 8)

Mycotoxin	Sorbent	pH 3 (%)	pH 5 (%)	pH 7 (%)
Deoxynivalenol (DON)	№1	65.3±6.9 ^{a}	61.2±2.8 ^{ab}	52.7±6.4 ^{bcd}
	№2	56.4±3.9 ^{abc}	53.9±5.2 ^{bcd}	45.5±4.9 ^{d}
	№3	60.1±3.8 ^{ab}	59.9±5.4 ^{ab}	48.8±5.0 ^{cd}
Fumonysin B1	№1	25.9±1.6 ^{a}	6.72±0.8 ^{b}	4.8±0.6 ^{c}
	№2	0.0±0.0 ^{f}	2.1±0.5 ^{e}	0.4±0.2 ^{f}
	№3	0.0±0.0 ^{f}	0.0±0.0 ^{f}	2.8±0.5 ^{d}
Zearalenone (ZEA)	№1	47.5±1.8 ^{a}	41.0±1.6 ^{ab}	47.3±1.8 ^{a}
	№2	37.1±1.6 ^{abc}	40.9±1.6 ^{ab}	28.4±1.7 ^{c}
	№3	26.5±1.4 ^{c}	36.5±1.9 ^{abc}	32.2±1.6 ^{bc}

Note: means followed by different letters within each mycotoxin differ significantly ($P < 0.05$; Tukey's test, comparison among 9 groups).

The highest level of Deoxynivalenol sorption was characteristic of sorbent No. 1 at pH 3 (65.3 ± 6.9%). With an increase in pH to 5 and 7, its sorption activity decreased slightly (61.2 ± 2.8 and 52.7 ± 6.4%, respectively). Sorbent No. 2 showed the lowest DON binding capacity at all pH levels (45.5–56.4%), while No. 3 occupied an intermediate position, maintaining moderate efficiency in all environments (48.8–60.1%).

Significant differences between sorbents were found. Sorbent No. 1 showed the highest sorption capacity for Fumonisin B1 at pH 3 (25.9 ± 1.6%), but its efficiency decreased sharply with increasing pH (to 6.72 ± 0.8 at pH 5 and 4.8 ± 0.6 at pH 7). Sorbents No. 2 and No. 3 showed very low or zero sorption in acidic and neutral environments, with isolated cases of weak activity (up to 2.8 ± 0.5%). Thus, only sorbent No. 1 provided clinically significant FB1 binding capacity, mainly at pH 3.

The sorption activity for zearalenone was different. Sorbent No. 1 provided consistently high values at pH 3 and 7 (47.5 ± 1.8 and 47.3 ± 1.8%), but showed a

decrease in efficiency at pH 5 (41.0 ± 1.6%). Sorbent No. 2 was characterized by moderate sorption at pH 3–5 and a significant decrease at pH 7 (28.4 ± 1.7%). The lowest values were obtained for No. 3, which showed limited ZEA binding capacity in all media (26.5–36.5%). Thus, sorbent No. 1 was the most effective for ZEA, especially at pH 3 and 7.

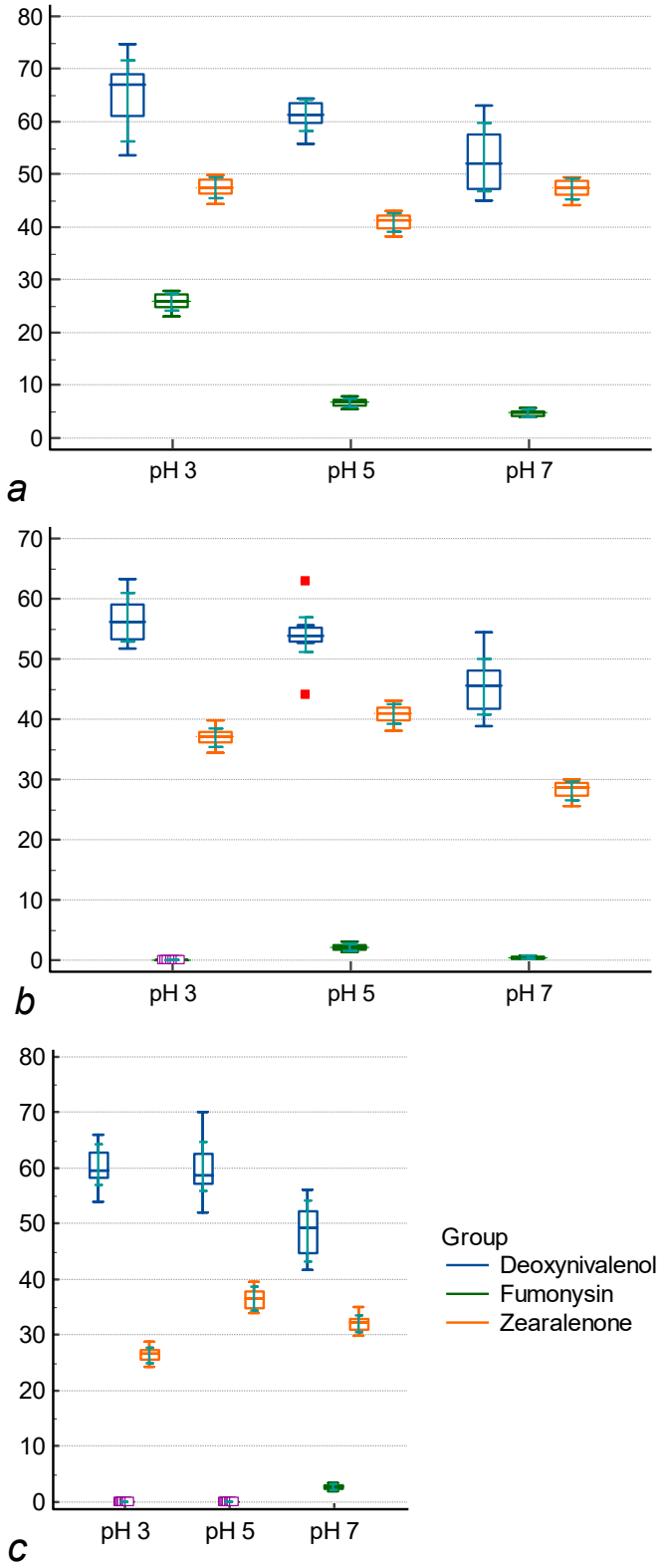

Figure 1 Absorption of mycotoxins in percent using sorbent 1 (a), sorbent 2 (b) and sorbent 3 (c): on the x-axis – percent (%); on the y-axis – the amount of bound mycotoxins at different pH values by each sorbent; small square – median, upper and lower rectangle borders – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n = 8

Sorbents No. 2 (20% MOS, 35% *β*-glucans) and No. 3 (20% MOS, 20% *β*-glucans) exhibited lower adsorption efficiency and a more pronounced pH dependence. For deoxynivalenol (DON), their adsorption capacity ranged from 45.5% to 60.1%, whereas for fumonisin B_1 (FB_1), it was minimal or nearly negligible. The adsorption of zearalenone (ZEA) by these formulations was also substantially lower (26.5–36.5%), particularly under neutral pH conditions.

The assessment of adsorption capacity toward aflatoxin B_1 revealed significant differences among the three tested binders, as well as a clear dependence of binding efficiency on pH. As shown in Table 2, sorbent No. 1 exhibited the highest adsorption capacity for aflatoxin B_1 across all tested pH conditions.

Sorbent No. 2 demonstrated slightly lower adsorption efficiency, ranging from 86.2 ± 1.6% to 93.3 ± 1.7%, with a noticeable decline at pH 5 and pH 7 compared to pH 3. The lowest adsorption capacity was observed for sorbent No. 3, with values ranging from 84.6 ± 1.6% to 90.0 ± 1.7%, showing a more pronounced dependence on pH.

Overall, sorbent No. 1 –characterized by the highest content of mannan-oligosaccharides (MOS, 33%) and *β*-glucans (25%) – can be considered the most versatile and effective formulation against a broad spectrum of mycotoxins. Its superior performance, particularly under acidic conditions typical of the animal gastrointestinal tract, supports its practical applicability for the prevention of mycotoxicoses.

Table 2 Comparison of three sorbents in terms of sorption capacity for Aflatoxin B1 at different pH levels (x ± SD, n = 8)

Mycotoxin	Sorbent	pH 3 (%)	pH 5 (%)	pH 7 (%)
Aflatoxin B1	№1	100±0.0 [a]	100±0.0 [a]	100±0.0 [a]
	№2	93.3±1.7 [b]	86.2±1.6 [d]	89.7±1.8 [c]
	№3	84.6±1.6 [d]	90.0±1.7 [c]	87.6±1.8 [cd]

Note: means followed by different letters within each mycotoxin differ significantly ($P < 0.05$; Tukey's test, comparison among 9 groups).

In our view, the high efficacy of Sorbent No. 1 can be attributed to the optimal ratio of *β*-glucans and mannan-oligosaccharides (MOS). This interpretation is consistent with the findings of **Feizy *et al.* (2025)**, who demonstrated that an optimized composition of bentonite, humic acids, and a *β*-glucan–mannan complex (70:10:20) significantly enhances adsorption efficiency ($P < 0.05$). This formulation achieved high detoxification rates, reaching 98.07% for aflatoxin B_1, 81.64% for ochratoxin A (OTA), 73.45% for zearalenone (ZEA), and 98.98% for deoxynivalenol (DON), thereby confirming a pronounced synergistic effect between inorganic and organic components.

These findings are in agreement with previous studies indicating that mycotoxin adsorption capacity is largely determined by chemical structure, polarity, and environmental conditions. According to **Kihal *et al.* (2022)**, aflatoxins exhibit the highest adsorption efficiency among major mycotoxins, whereas DON and fumonisin B_1 (FB_1) are less readily bound. Moreover, the efficacy of yeast cell walls increases markedly under acidic conditions, which can be explained by changes in the ionization state of the toxins and the activity of functional groups on the adsorbent surface. **Yiannikouris *et al.* (2006)** reported that *β*-D-glucans interact with mycotoxins through hydrogen bonding involving hydroxyl and lactone groups, thereby stabilizing the toxin-sorbent complex. Furthermore, the use of *Saccharomyces cerevisiae* cell walls (e.g., Agrimos) may confer additional immunomodulatory effects, as demonstrated by **Awaad *et al.* (2011)**, thereby enhancing the functional value of the preparation. The health benefits of yeast cell wall extracts for swine were further elucidated by **Kim *et al.* (2019)**. The research highlighted that YCW components, specifically β-glucans and MOS, function not only as mycotoxin adsorbents but also as potent immunomodulators. According to Kim, these polysaccharides enhance intestinal integrity by modulating the gut microbiota and promoting the expression of tight junction proteins, thereby mitigating the systemic inflammatory response often triggered by mycotoxin ingestion.

In addition to adsorption, contemporary research has explored biotransformation approaches, including the use of *Lactobacillus plantarum* (**Eiri *et al.*, 2024**) and ozone treatment (**Antos *et al.*, 2024**), the latter of which can reduce grain contamination by up to 100%. However, given their technological simplicity and economic feasibility, complex adsorbents such as Sorbent No. 1 remain the most practical and widely applicable strategy for the prevention of mycotoxicoses.

CONCLUSIONS

The obtained results indicate that a strategy based on combining inorganic silicate sorbents with yeast cell wall biopolymers provides a high degree of synergy and enhanced binding efficiency across a broad spectrum of mycotoxins.

The *in vitro* modeling results demonstrated that binder No. 1 (33% MOS and 25% β-glucans) exhibited the highest overall efficacy and versatility. This formulation achieved complete (100%) binding of aflatoxin B_1 across the entire tested pH range (3.0–7.0). This finding indicates exceptional stability of the sorbent–toxin complex under dynamic gastrointestinal conditions, particularly in the acidic environment of the stomach, which is critical for preventing the initial absorption of the toxin.

It was established that the adsorption capacity of the tested formulations for DON, FB_1, ZEA, and aflatoxin B_1 was significantly influenced by both the chemical nature of the mycotoxin and the pH of the medium ($P < 0.05$). For deoxynivalenol (DON) and zearalenone (ZEA), the highest adsorption values were observed for sorbent No. 1 at pH 3.0, whereas sorbents No. 2 and No. 3 (with lower MOS content) exhibited low and unstable activity.

The *in vitro* adsorption of fumonisin B_1 (FB_1) was observed exclusively for sorbent No. 1 and occurred predominantly under acidic conditions (pH 3.0); the other tested formulations did not demonstrate significant binding of this toxin. This finding highlights the importance of the specific composition of the organic fraction in binders for the effective adsorption of polar *Fusarium* metabolites.

Sorbents No. 2 and No. 3 exhibited lower efficacy and a pronounced dependence on pH, which may limit their practical applicability in the prevention of combined mycotoxicoses compared to the optimized composition of sorbent No. 1.

At the same time, it should be emphasized that the *in vitro* results reflect only the potential adsorption capacity of the tested formulations. In the actual gastrointestinal environment, detoxification efficiency may be influenced by additional factors, including feed matrix composition, enzymatic activity, microbiota status, and digesta passage rate. Therefore, the findings of this study provide a scientific basis for further *in vivo* investigations aimed at confirming the zootechnical efficacy of the developed binders.

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