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In Vitro Assessment of Chitosan-Based Nanoparticles Versus Glucomannan in Binding to Aflatoxin B1 in Poultry Ration

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Abstract

FUNGAL growth and its secondary metabolites have a profoundly negative impact on the commercial poultry industry, resulting in reduced feed intake, poor growth performance, hepatorenal toxicity, and increased mortality rates. The primary objective of this study is to develop a mycotoxin binder with anti-mycotoxin activity and nanoparticle properties capable of inhibiting fungal growth, adsorbing mycotoxins, and mitigating their toxic effects. Chitosan-based nanoparticles (CNPs) were synthesized and characterized, exhibiting an average particle size of 56.6 nm, a polydispersity index (PDI) of 0.4, and a positive surface charge of 32.3 mV. When inoculated into Vero cells, CNPs showed no cytotoxic effects compared to the control group. The sulforhodamine B (SRB) assay revealed a cell viability of 94.02% at a concentration of 100 µg/ml, with an IC50 exceeding 100 µg/ml. The high permeability, biocompatibility, biodegradability, and non-toxic nature of CNPs were assessed in vitro for their efficacy as a mycotoxin binder for aflatoxin B1 in contaminated broiler feed. Reduction activity was compared with chitosan and glucomannan by quantifying aflatoxin B1 levels using validated High-Performance Liquid Chromatography (HPLC) analysis. The results introduce chitosan-based nanoparticles (CNPs) as innovative mycotoxin binders with enhanced adsorptive capacity, biocompatibility, and biodegradability. CNPs showed an 81.5% reduction in aflatoxin B1 levels, outperforming chitosan (16.4%) and offering multifunctional applications, including antimicrobial and anti-mycotoxin effects. Compared to glucomannan (98.3% reduction), CNPs stand out for their versatility in mitigating fungal growth and toxin impact in poultry feed, paving the way for advanced strategies in the poultry industry.

Keywords: Mycotoxin Binders, HPLC, poultry, Chitosan-based nanoparticles.

Introduction

The Egyptian poultry industry faces considerable challenges due to microbial diseases and mycotoxicosis in poultry feed [1]. The phenomenon of mycotoxicosis was first discovered in 1960 when a Brazilian shipment of peanut meal, used as feed for poultry and domestic animals, resulted in the death of over 100,000 turkeys in the UK. The unknown etiological factor responsible was later identified as mycotoxins [2]. Mycotoxins are a category of mycotic derivative metabolites with a broad biological spectrum, depending on their structure, capable of inducing adverse effects on human and animal health [3]. Due to their small molecular weight, mycotoxins can infiltrate food products and contaminate raw materials such as wheat, sorghum, corn, and peanuts, producing toxigenic metabolites [4]. Egypt's poultry sector is heavily reliant on imported feed ingredients, which increases the risk of fungal contamination throughout the supply chain, including production, transportation, and market storage [5].

Among the nearly 500 known mycotoxins, aflatoxins (AFs) are considered the most hazardous to human and animal health. These secondary metabolites are primarily produced by fungi of the genus *Aspergillus*, including *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. These fungi thrive in tropical and subtropical climates characterized by high temperatures and

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humidity [6]. The most prevalent forms of aflatoxins are B1, B2, M1, G1, and G2, with Aflatoxin B1 recognized as the most toxic. It can cause adverse effects such as hepatotoxicity [7], teratogenicity, mutagenicity, and carcinogenicity in humans and animals [8]. Moreover, the International Agency for Research on Cancer (IARC) has classified Aflatoxin B1 as a Group I human carcinogen [9]. In broiler flocks, aflatoxicosis leads to critical issues such as reduced growth performance, alterations in organ weights, compromised immune responses, increased susceptibility to infections, vaccination failure, hepatorenal damage [10], and disruptions in protein metabolism due to its high affinity for proteins like albumin. AFB1 accumulates in tissues, contributing to contamination spread [11].

Various strategies have been proposed to mitigate mycotoxin contamination in feed chains, including physical separation, thermal eradication, microbial degradation, and chemical treatments [2]. Chemical adsorbents such as zeolites, activated charcoal, clays, chitosan polymers, montmorillonites treated with organic cations, sodium bentonite, hydrated sodium calcium, and aluminosilicate have demonstrated the ability to degrade, bind, and absorb toxins to alleviate their toxic effects [10]. Recently, there has been a global shift towards environmentally friendly toxin-binding agents [1]. One promising approach is the incorporation of binding agents into mycotoxin-contaminated feed [12].

Chitosan, a natural biodegradable and biocompatible polymer derived from the deacetylation of chitin, a cellulose-like carbohydrate, has emerged as a viable feed additive [13,14]. Chitosan exhibits immunostimulant, antioxidant, anticoagulant, and antitumor properties and can serve in diagnostics, vaccine preparation, and production enhancement. It has applications as an antibacterial, antiviral, antiparasitic, antifungal, and anti-mycotoxin agent [15].

Nanotechnology offers innovative solutions with broad applications and significant potential in the poultry industry [16]. Nano-prepared particles possess unique physical and chemical properties, including sizes averaging between 1–100 nm and a large surface area, rendering them more bioavailable, stable, and bioactive compared to bulk materials [17]. Nanotechnology provides effective control measures against mycotoxicosis [18]. Several studies have demonstrated the efficacy of chitosan-based nanoparticles (CNPs) as antifungal agents against specific toxigenic fungi [19, 20]. CNPs have shown promising binding capacities for various mycotoxins, including aflatoxin, zearalenone, fumonisin, and ochratoxin [21]. Research indicates a direct correlation between chitosan concentrations, inhibition of fungal growth such as *Aspergillus flavus*, and prevention of aflatoxin B1 production [22]. This study aims to prepare and characterize chitosan-based nanoparticles (CNPs) and evaluate their in vitro role as mycotoxin binders in poultry rations contaminated with Aflatoxin B1, comparing their efficacy to that of chitosan and glucomannan.

Material and Method

Reagents:

Chitosan was obtained from Sigma-Aldrich, USA. All sample preparation and extraction reagents were analytically pure from Merck (Sigma-Aldrich, Germany). Acetonitrile, methanol, water, and trifluoroacetic acid were HPLC grade (Fisher). Esterified glucomannan (EGM, MTB-100®) was obtained from IFT Corporation, Egypt. Phosphate Buffer Saline PBS at pH 7.4 consists of 0.2 g potassium chloride, 0.2 g potassium dihydro-gen phosphate, 1.16 g hydrogen, 8 g sodium chloride phosphate, and 900 mL deionized water. A certified reference solution of Aflatoxin B1 at 20 µg/mL in methanol (CRM44647, Supelco® Solutions from Merck) was utilized. An intermediate solution (1 µg/mL) was created in methanol by transferring 500 µL to a 10 mL volumetric flask and diluting with methanol to the mark. Blank poultry ration mycotoxins free obtained from the chemistry, toxicology, and feed deficiency department at AHRI, Doki, Giza.

Preparation of chitosan-based nanoparticles (CNPs):

Chitosan-based nanoparticles (CNPs) were prepared in the nanotechnology unit at the Animal Health Research Institute; chitosan-based nanoparticles (CNPs) were synthesized using the method of ionotropic-gelation, which relies on the electrostatic interaction between the negatively charged tripolyphosphate (TPP) and the positively charged amino groups of chitosan according to [23]. The process involves several steps: Chitosan Solution Preparation: Dissolving 3 g of chitosan in 6 ml of glacial acetic acid with 600 ml of acidified distilled water. The solution is stirred vigorously until transparent. pH Adjustment: Adjust the solution pH to 4.5-4.8 using NaOH and filtering to remove undissolved particles. TPP Solution: Dissolve 200 mg of TPP in 200 ml of distilled water. Dropwise Addition: Add the TPP solution dropwise at 2 ml/min to the chitosan solution while stirring continuously at room temperature for 2 hours. Sonication: the mixture for 10 minutes to aid in nanoparticle formation. Centrifugation and Washing: Centrifuging the solution at 12,000 rpm at 4°C for 15 minutes, repeating twice with washing. Dialysis and Lyophilization: The sediments are diluted with distilled water and lyophilized to obtain the final chitosan-based nanoparticles.

Characterization of chitosan-based nanoparticles (CNPs)

The average particle size and surface charge of the prepared nanoparticles were determined using a Zetasizer (Micotrac, Wave II, USA).

Cytotoxicity evaluation of chitosan-based nanoparticles (CNPs)

To evaluate the optimal concentration of the prepared nanocomposite formula, various concentrations of CNPs (0.01, 0.1, 1, 10, and 100 µg/ml) were inoculated into confluent Vero cells to determine their cytopathogenic effects (CPE) following the method described by Allam et al. [24]. The assay utilized sulforhodamine B (SRB), as outlined by Vichai and Kirtikara [25].

In vitro binding ability of chitosan-based nanoparticles in commercial broiler feed:

A 25 g batch of compounded broiler finisher feed was placed in a 250 ml Erlenmeyer flask. Culture material was added to achieve an Aflatoxin B1 toxin concentration of 100 ppb. Binders were incorporated into the flasks at a rate of 0.2%, while the control flasks contained untreated samples. Subsequently, 100 ml of citric acid-sodium phosphate buffer, adjusted to a pH of 6.5, was added to each flask. The contents were incubated at 37°C for 3 hours, followed by filtration and drying for 2 hours at the same temperature. The toxin was extracted and quantified, with the recovery rate expressed as a percentage. To evaluate Aflatoxin B1 binding in the various treatments, the difference in toxin content percentage between the initial and final stages of the trials for both binder-treated and control flasks was calculated using High-Performance Liquid Chromatography (HPLC). The binding percentage was determined by subtracting the toxin content percentage in control flasks from that of treated flasks, as described by Manafi et al. [26]. The formula used to calculate the adsorption percentage is:

Adsorption percentage = [(BT - ET) / BT * 100] - [(BC - EC) / BC * 100],

Where:

- BT = content of toxin in treated flasks at the beginning,
- ET = content of toxin in treated flasks at the end,
- BC = content of toxin in control flasks at the beginning, and
- EC = content of toxin in control flasks at the end.

Determination of Aflatoxin B1 in feed by HPLC:

To prepare your sample, combine 200 mL of 80% methanol with it to create a mixture. Stir the mixture vigorously for 3 minutes at high speed to ensure thorough blending. After blending, filter the solution through standard filter paper to remove larger particles. Next, dilute the filtered solution with water to lower the methanol concentration. Once diluted, filter the solution again using a glass microfiber filter for finer filtration. For the immunoaffinity cleanup (IAC), use AflaCLEAN™: Begin by pouring 10 mL of phosphate-buffered saline (PBS) out of each IAC to set them. Take 70 mL of the filtered sample previously and pass it through the IAC at a controlled flow rate of one drop per second. Afterward, clean the IAC by rinsing it with 10 mL of water to remove any remaining dirtiness. Apply a mild vacuum to dry the IAC and prepare it for the next steps. To elute (extract) the aflatoxins, pass 1.5 mL of methanol through the IAC, followed by another 1.5 mL of pure water to complete the elution process, as described by Khorrami et al. [27].

Chromatographic conditions:

Using the Agilent Technologies 1200 Infinity system (USA), 50 µL of the eluted samples were injected into an HPLC column (Inertsil ODS-4, 5 µm, 250 x 4.6 mm) maintained at 40ºC for optimal separation. The mobile phase was prepared by mixing water, methanol, and acetonitrile in a 60:20:20 (v/v/v) ratio, supplemented with 120 mg of potassium bromide and 350 µL of 4 M nitric acid per liter of the mixture. Fluorescence detection (FLD) was employed for the analysis of aflatoxins, with an emission wavelength of 365 nm and an excitation wavelength of 435 nm, ensuring precise measurement. The HPLC assay was validated in accordance with ICH guidelines [28], with a standard curve ranging from 1.0 to 100 ppb. The limits of detection (LOD) and quantification (LOQ) were calculated to meet sensitivity and accuracy requirements.

Statistical analysis

The data was analyzed using SPSS software (SPSS Inc., version 20.0) in Chicago, Illinois. For repeated group comparisons, a one-way analysis of variance along with an LSD post hoc test was used. The mean and standard deviation (SD) were used to express the data, and P-values less than 0.05 were considered statistically significant according to Kim [29].

Results

Characterization of chitosan-based nanoparticles (CNPs):

Morphological characters:

The zeta potential reveals that the obtained chitosan-based nanoparticles (CNPs) possess an average particle size of 56.6 nm, with a polydispersity index (PDI) of 0.4 and a positive surface charge of 32.3 mV, as depicted in Fig. 1. This parameter is essential for assessing the stability and behavior of nanoparticles in suspension. The

relatively small particle size of 56.6 nm is particularly advantageous for applications requiring fine particles, such as drug delivery or imaging. The particle size was determined using techniques like Dynamic Light Scattering (DLS), which offers precise measurements of dimensions. Smaller particles typically exhibit increased surface area-to-volume ratios, enabling improved interaction with biological systems and substrates, thereby enhancing their functionality in various applications

The polydispersity index (PDI):

The polydispersity index (PDI) value of 0.4 reflects the uniformity of particle sizes within the sample. A PDI closer to zero indicates higher particle size uniformity, whereas values approaching one suggest a broader size distribution. In this case, a PDI of 0.4 represents a moderately narrow size distribution, which is favorable for consistent nanoparticle behavior and performance. Uniform particle size is essential for predictable functionality, ensuring reproducibility in both research settings and practical applications.

The positive surface charge

The zeta potential of 32.3 mV signifies the electrostatic potential at the nanoparticle surface and plays a critical role in the stability of the suspension and its interaction with biological membranes. A high zeta potential whether negative or positive indicates strong electrostatic repulsion between particles, minimizing the likelihood of aggregation and resulting in a stable suspension where particles remain uniformly dispersed. Moreover, the positive surface charge enhances interactions between nanoparticles and negatively charged biological membranes, which is advantageous for applications such as targeted drug delivery systems.

The cytotoxicity of chitosan-based nanoparticles (CNPs):

Inoculation of Vero cells with chitosan-based nanoparticles (CNPs) demonstrated no cytopathic effects (CPE) after 72 hours compared to the control group. The cellular viability was assessed using the sulforhodamine B (SRB) assay, a colorimetric technique that evaluates cellular protein content as a measure of cell density. Conducted three days post-inoculation, the assay results revealed a cell viability percentage of 94.02% at a concentration of 100 µg/ml, with the half-maximal inhibitory concentration (IC50) exceeding 100 µg/ml. This indicates that higher concentrations would be required to inhibit 50% of cell viability, underscoring the low cytotoxicity of chitosan-based nanoparticles.

Invitro assessment of chitosan-based-nanoparticles (CNPs) as mycotoxin binder to contaminated feed ration:

HPLC results:

Aflatoxin B1 was identified at a retention time of 1.976 minutes. This retention time is a critical parameter in chromatography, as it reflects the duration taken for a compound to traverse the chromatographic system and reach the detector. Consistent retention times are essential for ensuring the reproducibility and reliability of the chromatographic method, as demonstrated in Fig. 2.

The standard curve for Aflatoxin B1 showed a linear relationship, with a correlation coefficient (R^2) of 0.99978. This statistical measure indicates the strength of the linear relationship between the concentration of Aflatoxin B1 and the detector response. A value close to 1 signifies an excellent linear relationship, suggesting that the detector response is directly proportional to the concentration of Aflatoxin B1, as illustrated in Fig. 3.

The assessment parameters for Aflatoxin B1 include the limits of quantification (LOQ) and the limit of detection (LOD). The LOQ is set at 0.2 ppb, which is the lowest concentration at which the compound can be reliably quantified. In contrast, the LOD is set at 0.07 ppb; this indicates the lowest concentration of the compound that can be detected, though it may not be quantified with acceptable precision and accuracy. The values of RSD% for intra-day and inter-day variation are 0.9 and 0.6 %, respectively.

Reduction level of Aflatoxin B1 in contaminated feed samples with different mycotoxin binders:

The reduction of Aflatoxin B1 levels observed after treatment with chitosan, chitosan-based nanoparticles, and glucomannan shows varying degrees of effectiveness among these adsorbents. Each treatment's effectiveness can be attributed to its unique properties and mechanisms of action. After treatment, Aflatoxin B1 levels decreased by 16.4% with chitosan, 81.5% with chitosan-based nanoparticles, and 98.3% with glucomannan, as illustrated in Table 1 and Fig. 4.

The varying degrees of reduction in Aflatoxin B1 levels by these treatments have significant implications for food safety and animal feed management. Chitosan, with its moderate reduction, can be used in situations where minimal contamination is present or in combination with other treatments. Chitosan-based nanoparticles, with their enhanced performance, offer a promising solution for more significant contamination scenarios. Meanwhile, glucomannan's near-complete

reduction of Aflatoxin B1 levels positions it as an ideal candidate for applications requiring stringent aflatoxin control.

Discussion

Mycotoxins and their secondary metabolites in poultry feed ingredients pose a significant threat to poultry farmers due to their adverse impacts on the health and performance of birds [30]. Among these, aflatoxins are particularly concerning, as they are associated with various detrimental outcomes, including impaired performance, reduced egg production, and compromised immune system functionality in poultry [31] (Saleemi et al., 2020). The growth of molds on feedstuffs, especially corn used in ration formulations, often leads to mycotoxin contamination [32]. Consequently, it is essential to propose dynamic mitigation strategies to ensure safety margins and prevent potential contamination of food products [33].

Several preventive protocols can be implemented to reduce mycotoxin levels in feed and minimize their hazardous effects. These include stringent quality control measures during manufacturing and storage, alongside the use of feed additives such as adsorbents, mycotoxin binders, enzymes, prebiotics, probiotics, and antioxidants [34]. Among the natural mycotoxin-binding agents suitable for the poultry industry, chitosan stands out. The inclusion of chitosan polymer nanoparticles in broiler diets enhances bioavailability and absorption compared to its conventional form, while also exhibiting potential anti-mycotoxin properties [18].

This study focused on the nanopreparation and characterization of chitosan particles, evaluating their toxicity levels on Vero cells. Additionally, in vitro assessments were conducted to evaluate their efficacy as a mycotoxin-binding agent for aflatoxin B1 in contaminated broiler feed, comparing their reduction activity to that of glucomannan. The preparation of polymeric chitosan-based nanoparticles involved inducing a chitosan solution (1% in acetic acid) and adding sodium tripolyphosphate (TPP) dropwise using the ionic gelation method, as described by Renu et al. [35].

Characterization results revealed that the nanoparticles had an average particle size of 56.6 nm, a polydispersity index (PDI) of 0.4, and a positive surface charge of 32.3 mV. This combination of small particle size, moderately narrow size distribution, and significant positive surface charge indicates favorable biocompatibility and cellular uptake, making the nanoparticles valuable for applications in the poultry industry. These findings align with previous studies by Hett [36] and Abd El-Ghany [37], which highlight the influence of nanoparticle size, solubility, shape, and charge on biological interactions and tissue permeability. These mechanisms improve cellular membrane uptake, facilitate targeted delivery to main sites, and ultimately enhance bioavailability [38].

Nanoparticles possess unique physicochemical properties that outperform bulk materials due to their high surface-to-volume ratio, increased surface reactivity, stability, bioactivity, bioavailability, controlled particle size, drug release, and site-specific targeting [39]. Their suspension stability further enhances interactions with biological systems, making them promising candidates for applications in therapeutics, diagnostics, vaccine production, and nutritional purposes [40].

The data demonstrated that chitosan-based nanoparticles (CNPs) did not exhibit any cytopathic effects (CPE) on Vero cells, nor did they induce observable toxicity at the cellular level when compared to the control group after 72 hours of inoculation. Furthermore, the Sulforhodamine B (SRB) assay revealed that at a concentration of 100 µg/ml, the viability of Vero cells was 94.02%, indicating minimal cytotoxicity. The half-maximal inhibitory concentration (IC50) was determined to be greater than 100 µg/ml. This assay was performed three days post-inoculation to assess cell viability, following the methodology outlined by Yang et al. [41]. These findings suggest that CNPs exhibit low cytotoxicity on Vero cells, making them promising candidates for various applications, including drug delivery systems, wound healing, and other therapeutic uses requiring high biocompatibility with Vero cells. Similar findings have been reported in studies by Sakurai et al. [42] and Al-Musawi et al. [43].

The in vitro evaluation of CNPs as mycotoxin binders in feed contaminated with aflatoxin B1 was conducted using High-Performance Liquid Chromatography (HPLC). Aflatoxin B1 was successfully separated with a retention time of 1.976 minutes, ensuring consistency and reliability in the chromatographic method. The precise retention time underscores the efficiency of the system in providing sharp, well-resolved peaks, crucial for accurate quantification of aflatoxin B1 [44]. HPLC is a widely utilized analytical technique for the separation, identification, and quantification of mycotoxins within mixtures [45]. Aflatoxins, which are harmful toxins found in livestock and poultry feed, require accurate and reliable quantification to mitigate their effects. HPLC ensures high recovery rates and accuracy in estimating aflatoxins extracted from feed ingredients [27].

The standard curve for Aflatoxin B1 exhibited linearity, with a correlation coefficient (R^2) of 0.99978. A value near 1 indicates an excellent correlation, demonstrating that the detector response is directly proportional to the concentration of Aflatoxin B1. These findings align with those of Smith and Johnson [44], who confirmed the method's reliability in producing accurate and consistent results across various concentration ranges. Similarly, this data corresponds with the conclusions of ICH [28], which emphasized that a high correlation coefficient ensures the method's applicability for a wide range of concentrations, making it versatile for analytical needs and reproducible in routine analyses. The limits of detection (LOD) and quantification (LOQ) for Aflatoxin B1 were determined, with an LOD of 0.07 ppb indicating high sensitivity to trace amounts, and an LOQ of 0.2 ppb highlighting the method's ability to reliably quantify low concentrations. These parameters are crucial for evaluating toxicity thresholds and health risks associated with Aflatoxin B1 contamination in food products, underscoring the method's suitability for food safety and quality control applications in compliance with regulatory standards [46]. The HPLC method for Aflatoxin B1 determination exhibits excellent precision, with intraday and inter-day RSDs of 0.9% and 0.6%, respectively. These low variability values indicate highly reproducible results within a day and across different days, meeting accepted analytical standards (i.e., below 2% RSD). This high level of precision confirms the suitability of the method for routine quality control and regulatory monitoring in complex sample matrices [28].

The reduction activity percentages of chitosan, chitosan-based nanoparticles, and glucomannan were statistically measured. The data revealed that chitosan, a natural polysaccharide, reduced Aflatoxin B1 levels by 16.4%. This modest reduction is attributed to chitosan's ability to form hydrogen bonds and electrostatic interactions with aflatoxin molecules. However, its larger particle size and lower surface area limit its adsorptive capacity. Additionally, the presence of competing substances within the feed matrix can impact on its binding efficiency, leading to a lower overall reduction of Aflatoxin B1 levels [47].

In contrast, chitosan-based nanoparticles achieved a remarkable 81.5% reduction in Aflatoxin B1 levels, demonstrating significantly enhanced adsorptive properties. This increased efficacy is primarily due to their larger surface area-to-volume ratio, providing more active binding sites, and their smaller size, which facilitates better penetration and interaction with aflatoxin molecules. This improved efficiency highlights the potential of nanotechnology in enhancing the performance of natural adsorbents [48]. Consistent with findings by Hassan et al. [49], the inhibition of fungal growth, such as *Aspergillus* species and their secondary metabolic toxins, was strongly associated with the release of hydrogen peroxide on the nanoparticle surface during the binding process. Key mechanisms for combating mycotoxins via nanotechnology include direct inhibition of fungal growth, adsorption of mycotoxins, and mitigation of their toxic impacts [18]. Chitosan-based nanoparticles have also demonstrated effective adsorption capacities for multiple mycotoxins, including aflatoxin, zearalenone, fumonisin, and ochratoxin [21].

Glucomannan, a polysaccharide derived from the konjac plant, showed the highest efficacy, achieving a 98.3% reduction in Aflatoxin B1 levels. Its superior binding capacity is attributed to the presence of multiple hydroxyl groups and higher molecular weight, which facilitate strong hydrogen bonding with aflatoxin molecules. Additionally, glucomannan's high water solubility enhances its ability to form stable complexes with aflatoxins, ensuring effective removal. These properties make it a highly effective adsorbent, as illustrated by Brown and Thompson [50].

Chitosan and nano-chitosan are considered superior to glucomannan as mycotoxin binders due to several intrinsic properties. As a naturally derived, biodegradable, and biocompatible cationic polymer, chitosan forms strong electrostatic interactions with the typically anionic mycotoxins, thereby enhancing its binding efficiency [19, 21]. When formulated into nanoparticles, chitosan exhibits a significantly increased surface area-to-volume ratio, which provides a greater number of active sites for toxin adsorption and improves dispersion and stability in complex feed matrices [13]. Additionally, nano-chitosan can be chemically modified to target a broader range of mycotoxins while offering antimicrobial benefits, making it a versatile tool for feed safety. In contrast, despite glucomannan's ability to bind aflatoxins, its high water solubility, limited binding versatility, and potential to interfere with nutrient absorption restrict its overall effectiveness under diverse commercial feed conditions [50]

Conclusions

The findings of this study emphasize the potential of chitosan-based nanoparticles (CNPs) as effective mycotoxin binders, characterized by their superior adsorptive capacity, biocompatibility, and biodegradability. With an 81.5% reduction in aflatoxin B1 levels, CNPs exhibit multifunctional benefits, including antimicrobial and anti-mycotoxin properties. Although glucomannan demonstrated a

higher aflatoxin reduction rate (98.3%), the additional capability of CNPs to inhibit fungal growth underscores their utility in enhancing the safety and performance of poultry feed. The study advocates further investigation into the application of CNPs within the poultry industry, recommending field evaluations and formulation optimization for scalable, cost-effective, and safe industrial usage.

Conflict of interest

According to the authors, there is no conflict of interest.

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Author contribution

All authors contributed to the design, analysis, observation, data collection, results, corrections, and oversight of the experimental work. They also wrote the original outline and performed statistical analysis of the results, with all authors revising and agreeing on the manuscript.

TABLE 1. Aflatoxin B1 (ppb) Concentration in feed after different treatments (n= 6).

Concentration of aflatoxin B1 in feed	Chitosan	Chitosan-based nanoparticles	Glucomannan
100 ppb	85	16.2	1.1
	88.4	21.1	3.2
	84.7	19.7	1
	81	17.8	1.6
	79	17.7	2.8
	80	15	2
Mean ± SD	83.0 ± 3.7	17.9 ± 2.2	2 ± 1

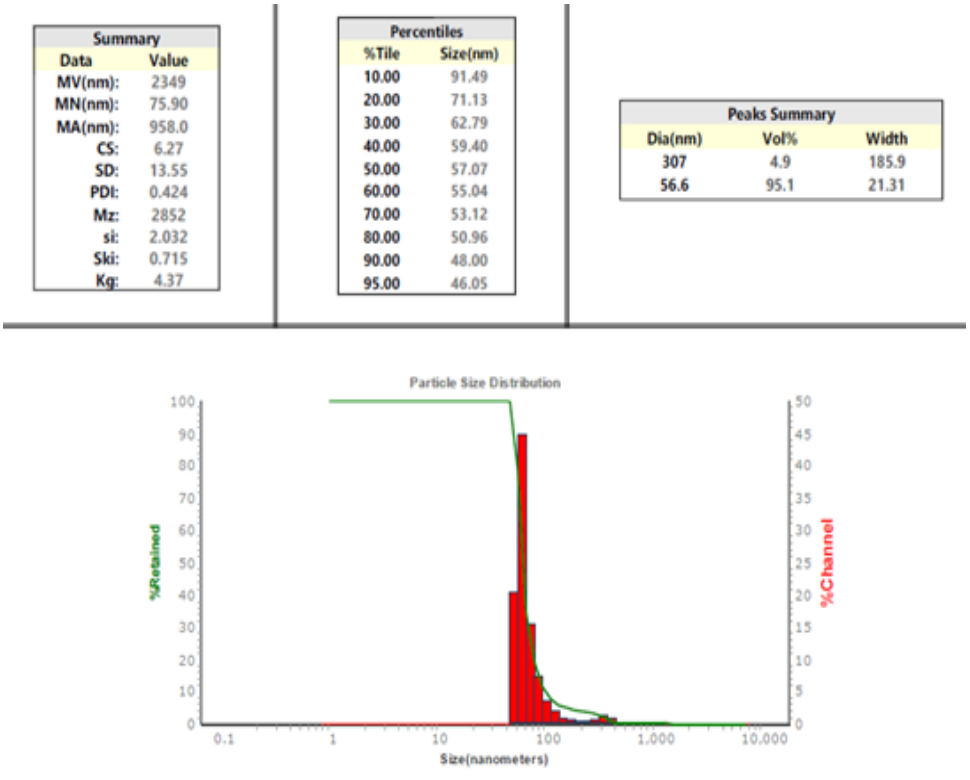

Fig. 1. Particle size analysis of chitosan-based nanoparticles using Zetasizer

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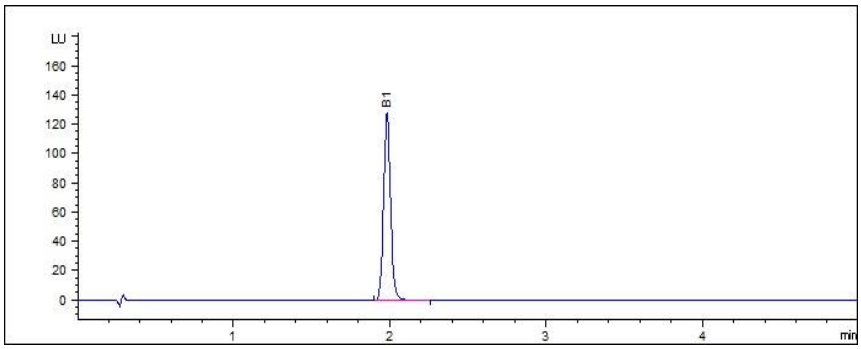

Fig. 2. The chromatogram shows Aflatoxin B1 (10 ppb) separation at 1.976 min.

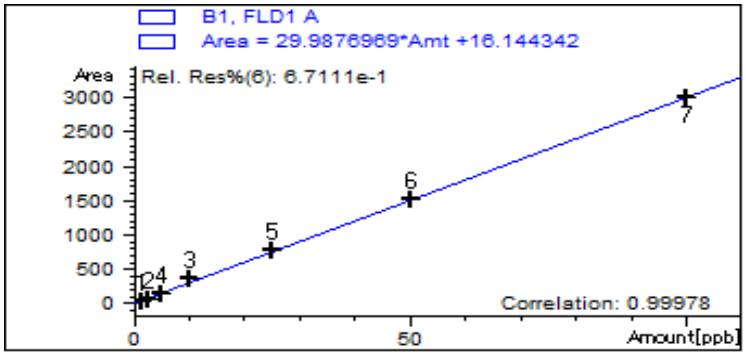

Fig. 3. The standard curve of Aflatoxin B1 ranges from 1.0 to 100 ppb

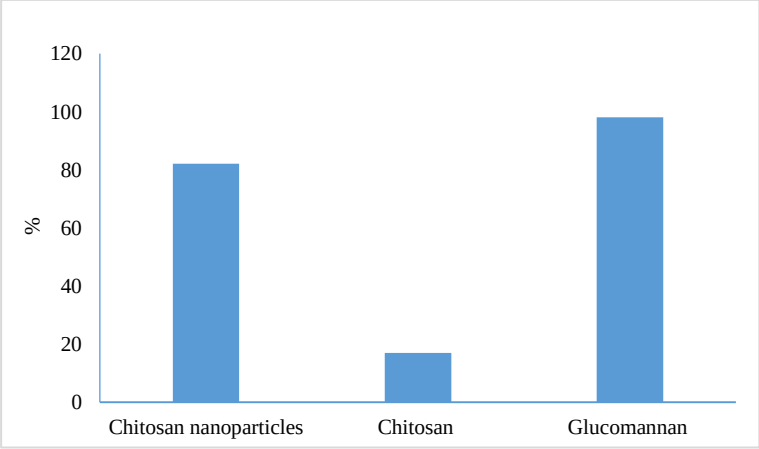

Fig. 4. Adsorption % of aflatoxin B1 (ppb) in feed after different treatments

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التقييم المختبري لجزيئات نانو الشيتوزان مقابل الجلوكومانان في الارتباط بالأفلاتوكسين B1 في علف الدواجن

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الملخص

يؤثر نمو الفطريات ومستقلباتها الثانوية سلبًا بشكل كبير على صناعة الدواجن التجارية، مما يؤدي إلى انخفاض استهلاك العلف، وضعف أداء النمو، وسمية كبدية كلوية، وزيادة معدلات الوفيات. الهدف الرئيسي من هذه الدراسة هو تطوير رابط للسموم الفطرية يتمتع بنشاط مضاد للسموم الفطرية وخصائص جسيمات نانوية قادرة على تثبيط نمو الفطريات، وامتصاص السموم الفطرية، وتخفيف آثارها السامة. تم تصنيع وتوصيف جسيمات نانوية قائمة على الشيتوزان (CNPs)، حيث أظهرت متوسط حجم جسيمات يبلغ 56.6 نانومتر، ومؤشر تعدد التشتت (PDI) يبلغ 0.4، وشحنة سطحية موجبة تبلغ 32.3 مللي فولت. عند تلقيحها في خلايا فيرو، لم تُظهر جسيمات CNPs أي آثار سامة للخلايا مقارنةً بالمجموعة الضابطة. أظهر اختبار سلفورودامين ب (SRB) حيوية الخلايا بنسبة 94.02% عند تركيز 100 ميكروغرام/مل، مع تجاوز IC50 لـ 100 ميكروغرام/مل. قُيِّمت نفاذية الجسيمات النانوية عالية النفاذية، والتوافق الحيوي، والتحلل الحيوي، وعدم سميتها في المختبر لفعاليتها كرابط للسموم الفطرية للأفلاتوكسين ب1 في علف دجاج التسمين الملوث. قورن نشاط الاختزال بالشيتوزان والغلوكومانان من خلال تحديد مستويات الأفلاتوكسين ب1 باستخدام تحليل كروماتوغرافيا السائل عالية الأداء (HPLC) المُعتمد. تُقدم النتائج الجسيمات النانوية القائمة على الشيتوزان (CNPs) كرابطات سموم فطرية مبتكرة ذات قدرة امتصاص مُحسَّنة، وتوافق حيوي، وقابلية تحلل حيوي. أظهرت CNPs انخفاضًا بنسبة 81.5% في مستويات الأفلاتوكسين B1، متفوقةً على الشيتوزان (16.4%)، ومُقدمةً تطبيقات متعددة، بما في ذلك تأثيرات مضادة للميكروبات والسموم الفطرية. وبالمقارنة مع الجلوكومانان (انخفاض بنسبة 98.3%)، تتميز CNPs بتعدد استخداماتها في الحد من نمو الفطريات وتأثير السموم في أعلاف الدواجن، مما يُمهد الطريق لاستراتيجيات متقدمة في صناعة الدواجن.

الكلمات الدالة: روابط السموم الفطرية، كروماتوغرافيا السائل عالي الأداء، الدواجن، الجسيمات النانوية القائمة على الشيتوزان.