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Mechanism of Action of Montmorillonite and Its Application in Poultry Production: Postprint

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Abstract

Montmorillonite (MMT) is a natural layered aluminosilicate mineral that can adsorb mycotoxins, heavy metals, and bacteria, repair and protect the gastrointestinal mucosa, and prevent diarrhea; additionally, modified MMT possesses antibacterial properties. Studies have shown that MMT can improve animal production performance, enhance product quality, and promote intestinal health. This article elaborates on the physicochemical properties, modification methods, main functions, and mechanisms of MMT, and reviews the latest research findings by domestic and foreign scholars on the application of MMT in poultry production.

Full Text

Functional Mechanisms of Montmorillonite and Its Application in Poultry Production

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Abstract

Montmorillonite (MMT) is a natural layered aluminosilicate mineral that exhibits multiple biological functions, including adsorption of mycotoxins, heavy metals, and bacteria, repair and protection of the digestive tract mucosa, and prevention of diarrhea. Additionally, modified MMT possesses antimicrobial properties. Research has demonstrated that MMT can improve animal production performance, enhance product quality, and promote intestinal health. This paper elaborates on the physicochemical properties and common modification methods of MMT, summarizes its primary functions and mechanisms of action, and reviews the latest research findings on the application of MMT in poultry production.

Keywords: montmorillonite; modification; mechanism; poultry; application

Introduction

With increasing global environmental pollution, various contaminants pose growing threats to livestock and human health. Environmental toxicants such as mycotoxins, bacteria, and heavy metals have attracted worldwide attention due to their insidious, latent, hazardous, and chronic nature. Developing control technologies to mitigate or eliminate the harm caused by these environmental toxicants to livestock and food safety represents a key research focus in feed science. Montmorillonite (MMT) is an aluminosilicate mineral with excellent ion exchange capacity, adsorption properties, and swelling capacity, enabling it to adsorb mycotoxins, heavy metals, and bacteria in the animal intestine while repairing and protecting the digestive tract mucosa and preventing diarrhea [1-4]. Furthermore, modified MMT exhibits antimicrobial effects [5]. Studies have shown that adding MMT and its modified products to animal diets can improve production performance, enhance product quality, and promote intestinal health [6-8]. This paper describes the main characteristics and common modification methods of MMT, summarizes its primary functions and mechanisms, and reviews the application effects of MMT in poultry production to provide a reference for its better utilization in the feed industry.

1. Main Characteristics of MMT

MMT is the primary component of bentonite and belongs to the 2:1 type layered aluminosilicate minerals, where each structural layer consists of two silicon-oxygen tetrahedral sheets connected at their apexes, sandwiching one aluminum-oxygen octahedral sheet connected at its edges [9]. Due to the presence of interlayer spaces, MMT possesses both internal and external surface areas, resulting in a large specific surface area. Its particles are extremely fine with numerous micropores distributed across the surface, providing substantial micropore volume and surface adsorption capacity [10]. Additionally, isomorphic substitution occurs within MMT crystal layers, where trivalent aluminum ions (Al^{3}) in the center of aluminum-oxygen octahedra are replaced by divalent magnesium ions (Mg^{2}), divalent zinc ions (Zn^{2}), or where tetravalent silicon ions (Si) in silicon-oxygen tetrahedra are replaced by Al^{3} . This generates permanent negative charges of varying strengths in the interlayer spaces. To maintain electrical neutrality, the crystal layers adsorb cations from the environment, enabling ion exchange and thus electrostatic adsorption capacity [11]. Due to its adsorptive properties, MMT can absorb polar water molecules into its interlayer spaces, where they contact exchangeable cations and undergo hydration reactions, expanding the interlayer spacing and increasing volume several-fold, thereby exhibiting swelling properties [9].

2. Modification Methods and Principles of MMT

To further enhance MMT performance, systematic modification studies have been conducted by researchers worldwide with promising results. Common modification methods include organic modification, inorganic modification, and composite modification.

2.1 Organic Modification

Utilizing the exchangeable nature of interlayer cations in MMT, organic modifiers can be introduced into the interlayer spaces to achieve organic modification. Modified MMT exhibits both hydrophilic and lipophilic properties, with further expanded interlayer spacing and increased porosity, significantly enhancing its adsorption performance. Additionally, cationic surfactants with bactericidal effects can be implanted into its crystal lattice, endowing modified MMT with antimicrobial efficacy. Currently, the most popular organic modifiers are quaternary ammonium salts and amine compounds, such as dodecyltrimethylammonium bromide (DTAB) and cetylpyridinium bromide (CPB). Zeng et al. [12] reported that DTAB-modified MMT adsorbed zearalenone (ZEA) with 8.9 times greater efficiency than natural MMT, while in vitro studies found that CPB-modified MMT exhibited strong bactericidal effects against Salmonella [13].

2.2 Inorganic Modification

Inorganic modification of MMT primarily utilizes its swelling and cation exchange properties to insert hydroxylated metal cations into the MMT lattice, propping apart the layers to form interlayer compounds. Upon further heating and dehydroxylation or dehydrogenation, these transform into stable pillar-like metal oxide clusters, creating new materials with layered pillar structures. This modification further increases interlayer spacing and micropore quantity and volume, resulting in stronger adsorption capacity. Daković et al. [14] found that zinc-loaded MMT bound aflatoxin B1 (AFB1) significantly more effectively than natural MMT, while Bekic et al. [15] reported that aluminum- and iron-loaded MMT showed superior adsorption of ZEA compared to natural MMT. Furthermore, antimicrobial cations can be introduced into MMT interlayer spaces to create MMT antimicrobial agents, with numerous in vitro and in vivo studies confirming the bacteriostatic and bactericidal effects of antimicrobial cation-modified MMT [16-20].

2.3 Composite Modification

Composite modification involves the simultaneous introduction of two or more organic and/or inorganic modification molecules or ions into MMT interlayer spaces, which jointly act on the pillar support to form flexible and rigid pillars, imparting superior properties to MMT. Currently, composite-modified MMT is mainly applied in industrial wastewater treatment, where it effectively adsorbs phenol and benzo[a]pyrene in sewage [21]. Mycotoxins are a class of stable low-molecular-weight toxic compounds similar to these pollutants, suggesting that composite-modified MMT may also adsorb mycotoxins, potentially with superior performance compared to singly-modified MMT. However, research on the application of composite-modified MMT for mycotoxin adsorption is rarely reported and warrants further investigation.

3. Main Functions and Mechanisms of MMT

3.1 Adsorption of Mycotoxins

Studies have shown that MMT can adsorb aflatoxins (AF) or AFB1 [22-23], ZEA [12], fumonisins [24], and T-2 toxin [7], thereby mitigating their harmful effects on animals. The mechanisms of mycotoxin adsorption by MMT are as follows: most mycotoxin molecules contain polar groups such as -OH and -NH or polarizable groups such as C=C and -C H , which can be adsorbed onto MMT' s porous structure through surface adsorption forces, electrostatic adsorption, and intermolecular forces. Mycotoxins with rigid planar molecular structures can enter MMT' s internal surfaces, further expanding adsorption sites while interacting with exchangeable metal ions in the interlayer spaces, making them difficult to desorb. This forms stable MMT-mycotoxin complexes that reduce or eliminate the bioavailability of mycotoxins, which are then excreted through the animal' s intestine [25].

3.2 Bacterial Adsorption and Bactericidal Effects

Natural MMT has essentially no bactericidal or bacteriostatic effects but can adsorb bacteria such as Escherichia coli and Salmonella [13,26]. MMT exhibits hydration swelling and dispersion characteristics; after swelling, the negatively charged plate surfaces connect with positively charged edges to form "carriage-like" suspensions with "gel-sol-gel" thixotropic properties. MMT can also exfoliate into sheet-like particles with sizes similar to bacteria and surface charges. These "carriage-like" suspended particles can "lock" bacteria inside the carriages, which are then cleared through intestinal peristalsis [26-27]. MMT modified with antimicrobial metal ions or cationic surfactants exhibits antimicrobial activity through the following mechanisms: most bacterial surfaces carry negative charges [28], while modified MMT carries positive charges, enabling electrostatic attraction and adsorption of bacteria [29]. Modified MMT surfaces are enriched with antimicrobial metal ions or cationic surfactants that can directly act on bacteria, increasing cell membrane permeability, causing nutrient leakage, altering bacterial morphology, and releasing intracellular enzymes. Additionally, they can cause the release of intracellular potassium ions (K), inhibiting the tricarboxylic acid cycle pathway of bacterial respiratory metabolism and leading to bacterial death [13,16].

3.3 Adsorption of Heavy Metals

MMT can adsorb heavy metals in feed, alleviating their toxic effects on animals. Adding bentonite to high-copper diets effectively reduced copper accumulation in sheep liver, mitigating the incidence and symptoms of copper toxicity [3]. Additionally, MMT adsorbs heavy metals from water, effectively treating heavy metal contamination in aquatic environments [30]. The adsorption of heavy metals by MMT primarily utilizes its cation exchange capacity, adsorption properties, and hydration swelling characteristics.

3.4 Prevention and Treatment of Diarrhea

Hu et al. [31] reported that zinc oxide-MMT (ZnO-MMT) was as effective as zinc oxide in preventing diarrhea in piglets, with the dosage reduced by fourfold. Song et al. [32] demonstrated that copper-loaded MMT (Cu-MMT) effectively alleviated diarrhea in weaned piglets, with efficacy comparable to chlortetracycline. Furthermore, calcium MMT (Ca-MMT) reduced the incidence of diarrhea in broilers [6]. MMT adsorbs and inhibits bacteria and toxins in the animal digestive tract, effectively blocking pathogen adhesion and reducing intestinal bacterial infection and translocation. Simultaneously, it can bind to mucus proteins in the digestive tract, increasing mucus quantity and improving its cohesion and flexibility [4]. Additionally, MMT has viscoplastic properties; its crystal layers can slide open and extend in the digestive tract, forming a continuous protective film that maintains the barrier function of the digestive tract mucosa, thereby preventing and treating diarrhea.

4. Application Effects of MMT in Poultry Production

4.1 Improving Production Performance

Numerous studies have shown that MMT can effectively mitigate the harmful effects of dietary mycotoxins on poultry and improve production performance. Yang et al. [7] reported that MMT significantly ameliorated the reduced body weight gain and increased feed conversion ratio caused by T-2 and HT-2 toxins in broilers. Adding Ca-MMT to AFB1-contaminated diets significantly increased broiler body weight, average daily feed intake, and feed conversion efficiency [33]. Ca-MMT also alleviated the adverse effects of Clostridium perfringens challenge alone or in combination with AF on broiler growth performance [6]. Moreover, MMT reduced the negative impacts of mycotoxins on the production performance of meat ducks [8] and laying hens [34]. MMT applied to normal diets (without mycotoxin contamination) also improved poultry production performance. Dietary supplementation with Ca-MMT increased laying rate by 10.21% and reduced feed-to-egg ratio by 5.63% in laying hens [35]. Adding ZnO-MMT to broiler diets significantly increased average daily gain and average daily feed intake while reducing feed conversion ratio, whereas natural MMT had no significant effect on broiler growth performance [17]. Dietary Cu-MMT significantly improved broiler average daily gain and reduced feed conversion ratio, while natural MMT showed no significant effect [18]. In summary, MMT can effectively intervene in the toxic effects of mycotoxins on poultry, with modified MMT showing superior effects on production performance compared to natural MMT. The improvement in animal production performance by MMT may be attributed to: (1) effective adsorption of dietary mycotoxins in the intestine, reducing their bioavailability; (2) adsorption or inhibition of intestinal bacteria and toxins, repair and protection of the digestive tract mucosa, and improvement of the intestinal microecological environment; and (3) enhanced gastrointestinal digestive enzyme activity and promotion of nutrient absorption [17]. Since the properties of modified MMT are substantially improved and the loaded substances themselves possess certain nutritional and immunological functions, they exert better overall effects.

4.2 Enhancing Product Quality

MMT can affect carcass composition in broilers. Adding natural or modified MMT to AFB1-contaminated diets significantly increased breast and thigh muscle percentages and eviscerated yield while reducing abdominal fat percentage to varying degrees [36]. MMT can effectively reduce the residues of mycotoxins and heavy metals in poultry products. Yang et al. [7] found that toxins could be detected in the head, muscle, small intestine, and liver tissues of broilers within 1 hour of consuming T-2 and HT-2 toxin-contaminated feed, whereas these toxins were undetectable or significantly reduced in broilers fed MMT-supplemented diets. Bentonite significantly reduced AFB1 residues in broiler liver [37], and Desheng et al. [38] reported that MMT significantly decreased fluorine and lead concentrations in broiler bones. Additionally, MMT improved certain egg quality traits by significantly increasing egg specific gravity, albumen height, and yolk proportion [39], while Ca-MMT showed a trend toward increasing zinc and manganese deposition in egg yolk [35]. With growing consumer concern for livestock product quality and food safety, these studies suggest that MMT can reduce toxic substance content in animal products and may enhance nutritional value. However, current research on MMT' s effects on livestock product quality remains limited, and further investigation is needed to determine whether it can produce safe, high-quality animal products.

4.3 Enhancing Antioxidant Capacity and Immune Function

MMT demonstrates excellent intervention against mycotoxin-induced oxidative stress. Prvulović et al. [23] reported that bentonite significantly alleviated AFB1-induced oxidative damage in broiler liver and kidney, markedly increasing antioxidant enzyme activity and reducing malondialdehyde content. Dietary Ca-MMT mitigated ZEA-induced oxidative damage in growing laying hens by significantly increasing serum antioxidant enzyme activity and decreasing malondialdehyde content [40]. Studies on MMT in normal diets also demonstrated enhanced antioxidant capacity [35]. However, research on the molecular mechanisms underlying MMT' s regulation of antioxidant function is currently lacking, and whether it improves antioxidant capacity by regulating relevant antioxidant signaling pathways and activating downstream antioxidant enzyme gene mRNA and protein expression requires further investigation. Immune organ indices are important indicators for evaluating poultry immune performance. Adding Ca-MMT to AFB1-contaminated diets significantly increased spleen and thymus indices in meat ducks [8], and Shi et al. [41] reported that sodium MMT alleviated the negative effects of AF on immune organ development in broilers. Immunoglobulins (Ig) A, IgG, and IgM are the primary immune molecules directly involved in humoral immune responses in poultry. Ca-MMT effectively reversed AFB1-induced immunosuppression in meat ducks by significantly increasing serum IgG and IgM content [8]. As the largest reticuloendothelial phagocytic system, the liver constitutes an important component of the immune system. MMT also intervenes in mycotoxin hepatotoxicity. Adding MMT to mycotoxin-contaminated diets significantly reduced serum alkaline phosphatase, aspartate aminotransferase, or alanine aminotransferase activities in laying hens [35], broilers [42], and turkeys [43]. Studies in broilers and turkeys found that bentonite significantly alleviated liver lesions caused by AFB1 [24,43-44], and MMT significantly inhibited T-2 and HT-2 toxin-induced hepatocyte apoptosis by regulating the p53 signaling pathway and modulating B-cell lymphoma-2 gene (Bcl-2) and Bcl-2-associated X protein (Bax) mRNA expression levels [7]. In summary, MMT can effectively alleviate mycotoxin-induced oxidative damage and immunosuppression in poultry and enhance antioxidant and immune performance when added to normal diets, though the underlying mechanisms require further investigation.

4.4 Improving Intestinal Health

MMT improves poultry intestinal health and maintains intestinal mucosal barrier function. Ca-MMT alleviated AFB1-induced intestinal mucosal injury in meat ducks by significantly increasing villus height and the villus height-to-crypt depth (V/C) ratio [8]. MMT also improved AFB1-induced histopathological changes in turkey intestines [43]. Xia et al. [18] reported that Cu-MMT significantly increased jejunal villus height and V/C ratio in broilers. Furthermore, Hu et al. [17] used the Ussing chamber system to study the effects of ZnO-MMT on intestinal permeability in broilers, finding that it significantly reduced mannitol permeability in the colon and inulin permeability in the ileum and colon while increasing colonic transepithelial electrical resistance. These studies demonstrate that MMT helps maintain the physical barrier function of the intestinal mucosa in poultry, possibly by alleviating damage to intestinal epithelial cells caused by mycotoxins and bacteria and by repairing and protecting the intestinal mucosa. Hu et al. [17] also found that ZnO-MMT significantly reduced Clostridium counts in the small intestine and ceca of broilers, while Cu-MMT significantly decreased harmful bacteria and increased beneficial bacteria in the broiler intestine [18]. Additionally, our research group using high-throughput sequencing technology found that Ca-MMT significantly increased the abundance of certain beneficial bacterial genera in the ceca of laying hens [45]. These findings indicate that MMT can optimize intestinal microbiota structure, which is closely related to its bacterial adsorption and inhibition properties. Currently, few studies have investigated MMT' s effects on the intestinal mucosal immune barrier in poultry. However, previous research on illite and zeolite showed that these minerals could inhibit inflammatory responses in broiler intestines by regulating Toll-like receptor signaling pathways [46] and significantly increase secretory immunoglobulin A (sIgA) and IgG content in broiler small intestinal mucosa, enhancing intestinal immunity [47]. Given the structural and property similarities between MMT and these silicate minerals, MMT likely also plays a role in maintaining the intestinal immune barrier in poultry, which deserves attention and investigation.

5. Summary

Due to its unique structure and physicochemical properties, MMT can adsorb various environmental toxicants, exhibit antimicrobial effects, and prevent diarrhea. As a feed additive, it can maintain intestinal barrier function, improve animal health status, enhance production performance, and upgrade product quality. Currently, MMT is primarily applied as a mycotoxin adsorbent in animal production. However, due to its excellent antimicrobial efficacy after appropriate modification, without inducing drug resistance or causing secondary infections, it can serve as a novel antimicrobial agent. Nevertheless, research on the mechanisms of MMT action in livestock production remains limited. Future systematic and in-depth studies should focus on: (1) exploring new modification technologies, particularly composite modification, to enhance MMT' s adsorption and antimicrobial properties, validated through in vitro and in vivo experiments; (2) investigating the appropriate supplementation levels of MMT in different animal diets, as it may adsorb nutrients in the digestive tract and excessive addition may cause side effects; (3) examining the effects and mechanisms of MMT on animals under normal, stress, or adverse conditions, with particular emphasis on its mechanisms in regulating intestinal health; (4) investigating the effects and mechanisms of combined application of MMT with probiotics, acidifiers, or enzymes in improving livestock intestinal health; and (5) studying the application effects of modified MMT as an antibiotic alternative in different animal diets. "Antibiotic-free animal production" represents an inevitable trend for the healthy development of global animal agriculture. With continuous advancement in MMT modification technology and antibiotic alternative research, MMT and its modified products will play an increasingly important role in future animal production.

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