Mechanism of Action of Montmorillonite and Its Application in Poultry Production (Postprint)

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Date: 2018-12-24T00:00:00+00:00

Abstract

Montmorillonite (MMT) is a natural layered aluminosilicate mineral possessing capabilities to adsorb mycotoxins, heavy metals, and bacteria, repair and protect gastrointestinal mucosa, and prevent and treat diarrhea; additionally, modified MMT exhibits antibacterial effects. Research demonstrates 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 scholars worldwide regarding 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, as well as repairing and protecting the digestive tract mucosa and preventing 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 modification methods of MMT, summarizes its primary functions and mechanisms,

and reviews the latest research findings on the application of MMT in poultry production both domestically and internationally.

Keywords: montmorillonite; modification; mechanism; poultry; application

1. Main Characteristics of MMT

MMT is the primary component of bentonite and belongs to the 2:1 type layered aluminosilicate minerals. Each layer of MMT consists of two silicon-oxygen tetrahedral sheets connected by shared vertices, with one aluminum-oxygen octahedral sheet sandwiched between them connected by shared 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 and substantial micropore volume, conferring strong surface adsorption capacity [10]. Furthermore, 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 intensity in the interlayers. To maintain electrical neutrality, the crystal layers adsorb cations from the environment, enabling ion exchange and thus electrostatic adsorption capacity [11]. Because of its adsorptive properties, MMT can draw polar water molecules into its interlayers, where they undergo hydration reactions with exchangeable cations, expanding the interlayer spacing and increasing volume several-fold, thereby exhibiting swelling characteristics [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 Organic modification exploits the exchangeability of interlayer cations in MMT to introduce organic modifiers into the crystal layers, achieving organic functionalization. Modified MMT exhibits both hydrophilic and lipophilic properties, with further expanded interlayer spacing and increased porosity, substantially improving adsorption performance. Additionally, cationic surfactants with bactericidal effects can be implanted into the 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 primarily utilizes the swelling and cation exchange properties of MMT to insert hydroxylated metal cations into the crystal 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 substantially 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. Moreover, antimicrobial cations can be introduced into MMT interlayers to create MMT-based 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 interlayers, which jointly act on the pillar support to form flexible and rigid pillars, conferring superior performance. Currently, composite-modified MMT is mainly applied in industrial wastewater treatment, where it effectively adsorbs phenol and benzo[a]pyrene from 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 composite-modified MMT for mycotoxin adsorption is rarely reported and warrants further investigation.

3. Primary Functions and Mechanisms

3.1 Mycotoxin Adsorption Studies have shown that MMT can adsorb aflatoxins (AF) [22] or AFB1 [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 , enabling adsorption onto MMT’ s porous structure through surface adsorption forces, electrostatic attraction, and intermolecular forces. Mycotoxins with rigid planar molecular structures can penetrate the internal surfaces, further expanding adsorption sites while interacting with exchangeable metal ions in the interlayers, making them difficult to desorb. This forms stable MMT-mycotoxin complexes that reduce or eliminate mycotoxin bioavailability, which are then excreted through the animal’ s intestinal tract [25].

3.2 Bacterial Adsorption and Bactericidal Effects Natural MMT has essentially no bactericidal or bacteriostatic efficacy 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 "house-of-cards" suspensions with thixotropic "gel-sol-gel" properties. MMT can also exfoliate into sheet-like particles with dimensions similar to bacteria and surface charges. These "house-of-cards" suspension particles can "lock" bacteria within the structure, 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 bacterial adsorption through electrostatic attraction [29]. Modified MMT surfaces are enriched with antimicrobial metal ions or cationic surfactants that directly affect bacteria by increasing cell membrane permeability, causing nutrient leakage, altering bacterial morphology, and releasing intracellular enzymes. Additionally, they can release bacterial potassium ions (K), inhibiting the tricarboxylic acid cycle pathway of bacterial respiratory metabolism and leading to bacterial death [13,16].

3.3 Heavy Metal Adsorption MMT can adsorb heavy metals from 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 heavy metal adsorption capacity of MMT primarily utilizes its cation exchange properties, adsorptive capacity, and hydration swelling characteristics.

3.4 Diarrhea Prevention and Treatment Hu et al. [31] reported that zinc oxide-montmorillonite (ZnO-MMT) prevented diarrhea in piglets as effectively as zinc oxide but at one-quarter the dose. Song et al. [32] demonstrated that copper-loaded MMT (Cu-MMT) effectively alleviated diarrhea in weaned piglets, with efficacy comparable to chlortetracycline. Furthermore, calcium montmorillonite (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 combines with mucus proteins in the digestive tract to increase mucus quantity and enhance its cohesion and flexibility [4]. Additionally, MMT exhibits plastic viscosity, allowing crystal layers to slide open and extend in the digestive tract, forming a continuous protective film that maintains digestive tract mucosal barrier function and prevents diarrhea.

4. Applications in Poultry Production

4.1 Improving Production Performance Numerous studies have demonstrated that MMT effectively mitigates the adverse effects of dietary mycotoxins on poultry and improves production performance. Yang et al. [7] reported that

MMT significantly ameliorated reduced body weight gain and increased feed-to-gain 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 detrimental effects of *Clostridium perfringens* challenge alone or combined with aflatoxin on broiler growth performance [6]. Moreover, MMT reduced the negative impacts of mycotoxins on production performance in meat ducks [8] and laying hens [34]. MMT application in normal diets (without mycotoxin contamination) also improved poultry production performance. Dietary Ca-MMT supplementation increased laying rate by 10.21% and reduced feed-to-egg ratio by 5.63% in laying hens [35]. Dietary ZnO-MMT significantly increased average daily gain and average daily feed intake while reducing feed-to-gain ratio in broilers, whereas natural MMT had no significant effect [17]. Dietary Cu-MMT significantly improved average daily gain and reduced feed-to-gain ratio in broilers, while natural MMT showed no significant effect [18]. In summary, MMT effectively intervenes in mycotoxin toxicity in poultry, with modified MMT demonstrating superior efficacy compared to natural MMT. The mechanisms underlying improved animal performance may include: (1) effective adsorption of dietary mycotoxins in the intestine, reducing their bioavailability; (2) adsorption or inhibition of intestinal bacteria and toxins, repairing and protecting digestive tract mucosa, and improving intestinal microecology; and (3) enhanced gastrointestinal digestive enzyme activity and nutrient absorption [17]. Since modified MMT exhibits substantially improved properties and the substances loaded onto MMT possess nutritional and immunological benefits, they exert superior effects.

4.2 Enhancing Product Quality MMT influences 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 effectively reduces residues of mycotoxins and heavy metals in poultry products. Yang et al. [7] found that broilers fed T-2 and HT-2 toxin-contaminated diets showed detectable toxin levels in head, muscle, small intestine, and liver tissues within 1 hour, whereas these toxins were undetectable or significantly reduced in MMT-supplemented groups. 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, significantly increasing egg specific gravity, albumen height, and yolk proportion [39], with Ca-MMT showing a trend toward increased zinc and manganese deposition in egg yolk [35]. As consumer concern for livestock product quality and food safety grows, these studies suggest that MMT can reduce toxic substance content in animal products and may enhance nutritional value. However, research on MMT' s effects on livestock product quality remains limited, and further investigation is needed to determine its role in producing safe, high-quality animal products.

4.3 Enhancing Antioxidant Capacity and Immune Function MMT effectively intervenes in 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, significantly increasing serum antioxidant enzyme activity and decreasing malondialdehyde content [40]. Studies applying MMT to normal diets also demonstrated enhanced antioxidant capacity [35]. Currently, research on the molecular mechanisms of MMT' s regulation of antioxidant function is lacking, and whether MMT improves antioxidant capacity by regulating relevant antioxidant signaling pathways and activating downstream antioxidant enzyme gene mRNA and protein expression requires further investigation. Immune organ index is an important indicator 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 montmorillonite alleviated the negative effects of aflatoxin on immune organ development in broilers. Immunoglobulins (Ig) A, IgG, and IgM are primary immune molecules directly involved in humoral immune responses in poultry. Ca-MMT effectively reversed AFB1-induced immunosuppression in meat ducks, significantly increasing serum IgG and IgM contents [8]. As the largest reticuloendothelial phagocytic system, the liver is a vital 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 showed that bentonite alleviated AFB1-induced liver lesions [24,43-44], and MMT significantly inhibited T-2 and HT-2 toxin-induced hepatocyte apoptosis by regulating the p53 signaling pathway and modulating mRNA expression levels of B-cell lymphoma-2 gene (Bcl-2) and Bcl-2-associated X protein (Bax) [7]. In summary, MMT effectively alleviates mycotoxin-induced oxidative damage and immunosuppression in poultry and enhances antioxidant and immune performance when added to normal diets, though the underlying mechanisms require further study.

4.4 Improving Intestinal Health MMT improves intestinal health and maintains intestinal mucosal barrier function in poultry. Ca-MMT alleviated AFB1-induced intestinal mucosal injury in meat ducks, significantly increasing villus height and 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. Using Ussing chamber systems, Hu et al. [17] investigated 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 intestinal mucosal physical barrier function, likely by alleviating damage to intestinal epithelial cells caused by mycotoxins and bacteria and by repairing and protecting intestinal mucosa. Hu et al. [17] also found that ZnO-MMT reduced *Clostridium* populations in the small intestine and ceca of broilers, while Cu-MMT decreased harmful bacteria and increased beneficial bacteria in the intestinal tract [18]. Furthermore, 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]. Thus, 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 intestinal mucosal immune barriers in poultry, but research on illite and zeolite suggests they can inhibit inflammatory responses in broiler intestines by regulating Toll-like receptor signaling pathways [46] and significantly increase secretory IgA (sIgA) and IgG contents in small intestinal mucosa to enhance intestinal immunity [47]. Given the structural and property similarities between MMT and these silicate minerals, MMT likely plays a role in maintaining intestinal immune barriers in poultry, warranting attention and investigation.

5. Summary

Due to its unique structure and physicochemical properties, MMT adsorbs various environmental toxins, exhibits antimicrobial effects, and prevents diarrhea. As a feed additive, it maintains intestinal barrier function, improves animal health status, enhances production performance, and improves product quality. Currently, MMT is primarily applied as a mycotoxin adsorbent in animal production. However, appropriately modified MMT demonstrates excellent antimicrobial efficacy without inducing drug resistance or causing secondary infections, making it a promising novel antimicrobial agent. Nevertheless, research on MMT' s mechanisms of 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 adsorptive and antimicrobial properties, validated through in vitro and in vivo experiments; (2) investigating optimal MMT supplementation levels in different animal diets, as excessive addition may adsorb nutrients in the digestive tract and cause side effects; (3) examining MMT' s effects and mechanisms under normal, stress, or adverse conditions, with emphasis on its mechanisms regulating intestinal health; (4) investigating the effects and mechanisms of combined application of MMT with probiotics, acidifiers, or enzymes to improve intestinal health in livestock; and (5) studying the application effects of modified MMT as an antibiotic alternative in different animal diets. Antibiotic-free animal production is 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 increasingly important roles in future animal production.

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