Effects of Ultrafine Ground Attapulgite on Broiler Meat Quality Postprint

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Abstract

This experiment aimed to investigate the effects of ultrafine pulverized attapulgite on muscle quality in broiler chickens. A total of 192 one-day-old Arbor Acres broiler chickens were selected and randomly divided into 3 groups, with 8 replicates per group and 8 birds per replicate. The control group was fed a basal diet, while the two experimental groups were fed experimental diets supplemented with 1% conventionally ground attapulgite and 1% ultrafine pulverized attapulgite, respectively, for a 42-day experimental period. The results showed that: compared with the control group, dietary supplementation with either conventionally ground attapulgite or ultrafine pulverized attapulgite had no significant effect on the proximate chemical composition of broiler muscle ($P>0.05$); dietary supplementation with ultrafine pulverized attapulgite significantly reduced the contents of chromium and cadmium in thigh muscle ($P<0.05$), significantly increased the iron content in breast muscle ($P<0.05$), and significantly decreased the redness values of both breast and thigh muscles as well as the yellowness value of breast muscle ($P<0.05$); dietary supplementation with conventionally ground attapulgite only significantly reduced the chromium content in thigh muscle and the redness value of breast muscle ($P<0.05$). Compared with the control group and the conventionally ground attapulgite group, the ultrafine pulverized attapulgite group showed significantly increased glutathione peroxidase activity in breast muscle ($P<0.05$). It can be concluded that dietary supplementation with ultrafine pulverized attapulgite can reduce toxic heavy metal residues in muscle, affect muscle color, and improve the antioxidant capacity of breast muscle, with effects superior to those of conventionally ground attapulgite.

Full Text

Effects of Ultra-Fine Grinding Palygorskite on Muscle Quality of Broilers

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Abstract

This experiment investigated the effects of ultra-fine grinding palygorskite on muscle quality of broilers. A total of 192 one-day-old Arbor Acres broilers were randomly divided into 3 groups with 8 replicates of 8 birds each. Broilers in the control group were fed a basal diet, while those in the two experimental groups were fed the basal diet supplemented with 1% palygorskite in either regular grinding or ultra-fine grinding form for 42 days. The results showed that compared with the control group, neither regular grinding nor ultra-fine grinding palygorskite supplementation significantly affected the muscular macrochemical components of broilers ($P>0.05$). However, ultra-fine grinding palygorskite supplementation significantly decreased chromium (Cr) and cadmium (Cd) contents in the thigh muscle ($P<0.05$), significantly increased iron (Fe) content in the breast muscle ($P<0.05$), and significantly decreased redness values in both breast and thigh muscles as well as yellowness value in the breast muscle ($P<0.05$). Regular grinding palygorskite supplementation only significantly reduced Cr content in the thigh muscle and redness value in the breast muscle ($P<0.05$). Additionally, broilers in the ultra-fine grinding palygorskite group exhibited significantly higher glutathione peroxidase activity in the breast muscle compared with the other two groups ($P<0.05$). These findings suggest that dietary supplementation with ultra-fine grinding palygorskite can reduce toxic heavy metal residues, affect muscle color, and enhance the antioxidant capacity of breast muscle, with more pronounced effects than regular grinding palygorskite.

Key words: ultra-fine grinding; regular grinding; palygorskite; broilers; macrochemical components; mineral accumulations; muscle quality; antioxidant capacity

Providing safe and high-quality animal-derived food for consumers is the primary goal of animal production, and relevant technical measures to ensure animal product safety have become a key research focus. Palygorskite is a hydrated magnesium-aluminum silicate clay mineral and the main component of palygorskite clay. Its unique fibrous crystal structure and channel-like microstructure endow palygorskite with excellent adsorption and ion-exchange properties [1]. In vitro studies have demonstrated that palygorskite can effectively adsorb heavy metal ions from contaminated water and soil [2-3], and it has been

widely applied as a heavy metal adsorbent in wastewater treatment, medicine, and chemical industries [4]. In animal production, Cheng et al. [5] found that dietary supplementation with regular grinding palygorskite reduced lead (Pb) residues in broiler muscle. Cheng et al. [6] reported that adding 1% regular grinding palygorskite to Pb-contaminated diets decreased Pb and copper (Cu) contents in the liver, kidney, and breast muscle of broilers. Zhang et al. [7] also reported that dietary palygorskite supplementation reduced cadmium (Cd) deposition in the muscle of blunt snout bream. Furthermore, dietary palygorskite supplementation can promote animal growth and improve intestinal health and antioxidant capacity [8-9], with an optimal inclusion level of 1% [5,9-10].

Ultra-fine grinding technology is a process that uses mechanical or fluid dynamic methods to pulverize large particles into micrometer or even nanometer-sized particles [11]. Compared with regular grinding, ultra-fine grinding products exhibit physicochemical characteristics such as uniform particle size distribution, significantly increased specific surface area, and stronger surface adsorption capacity [12]. Clay minerals possess good adsorption properties, which are influenced by particle size—the smaller the particle size, the larger the specific surface area and the stronger the adsorption capacity [13]. Zhao et al. [14] demonstrated in vitro that ultra-fine grinding treatment significantly enhanced the heavy metal removal capacity of palygorskite. Berhane et al. [15] also found that as the particle size of palygorskite-montmorillonite complexes decreased, their adsorption capacity for compounds such as the antibiotic ciprofloxacin continuously increased.

Currently, regular grinding palygorskite products are predominantly used in animal production. Our previous studies have shown that regular grinding palygorskite supplementation can reduce toxic heavy metal deposition in muscle [5-7] and may also affect meat color and quality [5-6]. However, no studies have reported the effects of dietary ultra-fine grinding palygorskite on broiler muscle quality. Therefore, this experiment was conducted to investigate the effects of dietary ultra-fine grinding palygorskite on the macrochemical composition, mineral deposition, meat quality, and antioxidant function of broiler muscle, aiming to provide a reference for the rational application of ultra-fine grinding palygorskite in broiler diets.

1.1 Experimental Materials

The regular grinding palygorskite used in this experiment was purchased from Jiangsu Huida Mining Technology Co., Ltd. A portion of the regular grinding palygorskite was subjected to ultra-fine grinding using an ultra-fine grinding classifier (TC-40 model, Nanjing Longli Tianmu Ultra-Fine Powder Technology Co., Ltd.) to produce ultra-fine grinding palygorskite. Particle size analysis of the two grinding treatments was performed using a laser particle size analyzer (MASTERSIZER 2000 model, Malvern Instruments Ltd., UK), and the results are presented in Table 1 .

1.2 Experimental Design

A total of 192 one-day-old Arbor Acres (AA) broilers, with equal numbers of males and females, were randomly allocated into 3 groups with 8 replicates of 8 birds each. The broilers were housed in multi-tier cages, with each cage representing one replicate and similar body weight across replicates. The control group was fed a basal diet, the regular grinding palygorskite group received the basal diet supplemented with 1% regular grinding palygorskite, and the ultra-fine grinding palygorskite group received the basal diet supplemented with 1% ultra-fine grinding palygorskite. The experiment was conducted at Nanjing Kangxin Poultry Industry Co., Ltd., in a closed chicken house. The experimental period lasted 42 days, during which broilers had ad libitum access to feed and water, 24-hour continuous lighting, and followed a conventional immunization program. The composition and nutrient levels of the basal diet are shown in Table 2 .

1.3 Experimental Instruments

The following instruments were used in this study: an HI-9125 portable pH meter (HANNA Instruments, Italy); a CR-10 colorimeter (Konica Minolta, Japan); a ZKSY-600 intelligent thermostatic water bath (Shanghai Pudong Rongfeng Scientific Instrument Co., Ltd.); an EHD-36 heating block (Beijing LabTech Instruments Co., Ltd.); an Optima-2100 DV inductively coupled plasma mass spectrometer (ICP-MS) (Perkin Elmer, USA); a 5804-R refrigerated centrifuge (Eppendorf, Germany); and a PK-02200D homogenizer (Pro Science, USA).

1.4 Sample Collection

On day 42 of the experiment, one rooster with body weight close to the replicate average and in good health was selected from each replicate and slaughtered by exsanguination from the carotid artery. After dissection, the left portion of breast and thigh muscles were collected in self-sealing bags and stored at -20 °C for further analysis. Simultaneously, the entire right breast and thigh muscle samples were collected and stored at 4 °C for meat quality determination.

1.5.1 Determination of Muscular Macrochemical Components

The moisture, crude fat, and crude protein contents in muscle were determined according to the national standards: "Determination of Moisture in Foods" [16] (GB 5009.3-2016, direct drying method), "Determination of Fat in Foods" [17] (GB 5009.6-2016, Soxhlet extraction method), and "Determination of Protein in Foods" [18] (GB 5009.5-2016, Kjeldahl method), respectively.

1.5.2 Determination of Muscular Mineral Element Contents

The digestion of breast and thigh muscle samples was performed according to the method of Yang et al. [19]. Approximately 2 g of muscle sample was accurately

weighed and placed in a 50 mL flat-bottom cylindrical digestion tube. After adding 10 mL of mixed nitric acid-perchloric acid (V/V = 4:1), the sample was left to stand overnight and then digested in a heating block until clear. The digested solution was cooled, filtered into a 25 mL volumetric flask, and the contents of zinc (Zn), iron (Fe), magnesium (Mg), Cu, Cd, and chromium (Cr) were determined using ICP-MS. The heating block program was set as follows: 90 °C for 30 min, 120 °C for 30 min, 160 °C for 30 min, and 180 °C for 180 min. The ICP-MS operating conditions were: RF power 1,550 W; plasma gas flow 12 L/min; auxiliary gas flow 0.2 L/min; nebulizer gas flow 0.55 L/min; and sample uptake rate 1.0 mL/min.

1.5.3 Determination of Muscle Quality Parameters

Muscle pH was measured at 45 min post-slaughter and after 24 h refrigeration at 4 °C using a pH meter, with three measurements taken per sample and averaged. Color values (lightness L*, redness a*, and yellowness b*) were measured at 45 min post-slaughter using a colorimeter, with three measurements taken per sample and averaged. Drip loss was determined according to the method of Wang Xiaoming [20]. After slaughter, a 10 g muscle sample (W1) was weighed and suspended with a paperclip from a wire in a disposable paper cup, ensuring the sample did not contact the cup wall and that muscle fiber direction was parallel to gravity. The cup was placed in a self-sealing bag, sealed, and stored at 4 °C. The sample was removed at 24 h and 48 h, surface fluid was gently blotted with filter paper, and the sample was reweighed (W2, W3). Drip loss was calculated as: Drip loss 24 h (%) = [(W1 - W2)/W1] × 100; Drip loss 48 h (%) = [(W1 - W3)/W1] × 100. Cooking loss was determined according to the method of Wang Xiaoming et al. [20]. Muscle samples collected after slaughter were aged at 4 °C for 24 h, and a 10 g sample (W4) was weighed and placed in a self-sealing bag. A glass thermometer was inserted into the center of the muscle, the bag was sealed, and the sample was heated in a water bath until the core temperature reached 70 °C, which was maintained for 20 min. After cooling, the sample was dried and reweighed (W5). Cooking loss was calculated as: Cooking loss 48 h (%) = [(W4 - W5)/W4] × 100.

1.5.4 Determination of Muscular Antioxidant Capacity

Tissue homogenate preparation: 0.3 g of muscle sample was mixed with ice-cold sterile physiological saline at a ratio of 1:9 (mass/volume) and homogenized. The homogenate was centrifuged at 3,500 r/min for 10 min at 4 °C, and the supernatant was collected, aliquoted, and stored at -20 °C for analysis. The supernatant was used to determine total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, malondialdehyde (MDA) content, and total antioxidant capacity (T-AOC) according to the kit instructions. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Experimental data were initially processed using Excel 2010 and analyzed using SPSS 20.0 software. One-way ANOVA was performed, and Duncan' s multiple comparison test was used to examine significant differences among groups. Differences were considered significant at $P<0.05$. Results are expressed as mean ± standard error (SE).

2.1 Effects of Ultra-Fine Grinding Palygorskite on Muscular Macrochemical Components of Broilers

As shown in Table 3 , compared with the control group, dietary supplementation with either regular grinding or ultra-fine grinding palygorskite did not significantly affect the moisture, crude fat, or crude protein contents in breast and thigh muscles of broilers ($P>0.05$).

2.2 Effects of Ultra-Fine Grinding Palygorskite on Muscular Mineral Accumulations of Broilers

As shown in Table 4 , compared with the control group, ultra-fine grinding palygorskite supplementation significantly decreased Cd and Cr contents in the thigh muscle ($P<0.05$) and significantly increased Fe content in the breast muscle ($P<0.05$). Regular grinding palygorskite supplementation only significantly reduced Cr content in the thigh muscle ($P<0.05$). Furthermore, ultra-fine grinding palygorskite supplementation significantly increased Fe content in muscle compared with regular grinding palygorskite supplementation ($P<0.05$).

2.3 Effects of Ultra-Fine Grinding Palygorskite on Muscle Quality of Broilers

As shown in Table 5 , compared with the control group, ultra-fine grinding palygorskite supplementation significantly decreased redness values in both breast and thigh muscles and yellowness value in the breast muscle ($P<0.05$). Regular grinding palygorskite supplementation also significantly reduced redness value in the breast muscle ($P<0.05$). However, neither ultra-fine grinding nor regular grinding palygorskite supplementation significantly affected pH values (at 45 min and 24 h), drip loss (at 24 h and 48 h), cooking loss, or lightness values in breast and thigh muscles of broilers ($P>0.05$).

2.4 Effects of Ultra-Fine Grinding Palygorskite on Muscular Antioxidant Capacity of Broilers

As shown in Table 6 , GSH-Px activity in the breast muscle of the ultra-fine grinding palygorskite group was significantly higher than that in the control and regular grinding palygorskite groups ($P<0.05$). Compared with the control group, T-AOC in the breast muscle of the ultra-fine grinding and regular grinding palygorskite groups increased by 53.85% and 23.08%, respectively, but these

differences were not significant (P>0.05).

3 Discussion

3.1 Effects of Ultra-Fine Grinding Palygorskite on Muscular Macrochemical Components of Broilers Meat is rich in protein, fat, minerals, and other nutrients, serving as a major source of high-quality protein for humans [21]. Muscle chemical composition directly affects its nutritional characteristics, with moisture, crude fat, and crude protein typically considered as macrochemical components [22]. Pryvulovuc et al. [23] reported that dietary supplementation with 0.5% hydrated aluminosilicate (mainly montmorillonite) in finishing pigs significantly increased moisture content and decreased crude fat content in the longissimus dorsi muscle. In contrast, the present study showed that dietary supplementation with either regular grinding or ultra-fine grinding palygorskite did not significantly affect moisture, crude protein, or crude fat contents in broiler muscle. This discrepancy may be related to differences in animal species, clay mineral types and inclusion levels, feed management, and environmental conditions.

3.2 Effects of Ultra-Fine Grinding Palygorskite on Muscular Mineral Accumulations of Broilers Toxic heavy metals such as Cr and Cd can accumulate in animals after ingestion and absorption, posing a threat to human health through the food chain. Kong et al. [24] demonstrated in vitro that palygorskite can effectively adsorb heavy metal elements including Cd, Cr, and nickel (Ni) from solution. The present results showed that both regular grinding and ultra-fine grinding palygorskite supplementation reduced Cr residues in broiler thigh muscle, consistent with the findings of Cheng et al. [5-6] and Zhang et al. [7]. Compared with the regular grinding palygorskite group, ultra-fine grinding palygorskite supplementation significantly reduced Cd residues in thigh muscle. Zhou et al. [25] reported that fine-particle zeolite reduced Pb content in muscle more effectively than coarse-particle zeolite. Lin [26] found that ultra-fine grinding montmorillonite exhibited superior adsorption capacity for heavy metals Cu and Pb compared with raw montmorillonite under different pH conditions in vitro, due to its increased specific surface area. Therefore, the superior effect of ultra-fine grinding palygorskite on improving muscular mineral accumulation observed in this study may be attributed to its increased specific surface area.

Palygorskite is a natural alkaline earth magnesium-aluminum silicate mineral. As it passes through the animal digestive tract, mineral elements such as Fe contained in palygorskite can be released under acidic digestive fluid and absorbed by the animal [27]. Zhang et al. [7] reported that dietary supplementation with 2% regular grinding palygorskite increased Fe content in plasma and muscle of blunt snout bream. Similar results were obtained in the present study, and the effect of ultra-fine grinding palygorskite on increasing Fe content in breast muscle was superior to that of regular grinding palygorskite. This may be because

the smaller particle size of ultra-fine grinding palygorskite allows more complete contact with digestive fluid, promoting Fe release and facilitating its absorption and utilization, thereby increasing Fe content in breast muscle. Research has also shown that the saturated adsorption capacity of palygorskite for metal ions follows the order: Cr6+ > Cd2+ > Fe3+ [28-29]. Thus, ultra-fine grinding palygorskite reduced the residues of heavy metal elements Cr and Cd in muscle while increasing Fe deposition.

3.3 Effects of Ultra-Fine Grinding Palygorskite on Muscle Quality of Broilers Meat eating quality reflects the economic value of meat products, with evaluation indices including water-holding capacity, pH value, and color [21]. Meat color is a primary factor influencing consumer purchase decisions and holds significant commercial importance [30]. Cheng et al. [5] reported that dietary supplementation with 1% palygorskite decreased redness and yellowness values in breast muscle and yellowness value in thigh muscle without affecting other meat quality parameters. The present study demonstrated that both ultra-fine grinding and regular grinding palygorskite supplementation reduced redness and yellowness values in broiler muscle to varying degrees, consistent with the above findings. Corn-soybean meal diets for broilers contain natural carotenoids such as zeaxanthin, lutein, and carotene [31], which can affect carcass color [32]. In vitro experiments have proven that palygorskite can effectively adsorb pigments from edible oil [33-34]. Therefore, the reduction in muscle redness and yellowness values observed in this study may be related to the adsorption of pigments by palygorskite. Moreover, ultra-fine grinding palygorskite had a more significant effect on muscle color than regular grinding palygorskite, possibly because the ultra-fine grinding process increased the effective adsorption area of palygorskite particles, thereby enhancing its adsorption capacity for dietary pigments. Additionally, the present results showed that neither ultra-fine grinding nor regular grinding palygorskite supplementation significantly affected water-holding capacity or pH of breast and thigh muscles, consistent with the findings of Kavan et al. [35]. However, Yang et al. [36] reported that dietary palygorskite supplementation significantly increased muscle pH at 45 min and decreased drip loss at 48 h in growing-finishing pigs. The inconsistent reports on the effects of palygorskite on muscle quality may be related to animal species, palygorskite source, and inclusion level.

3.4 Effects of Ultra-Fine Grinding Palygorskite on Muscular Antioxidant Capacity of Broilers Animals rely on their endogenous antioxidant defense system to scavenge excess reactive oxygen species and prevent oxidative damage. Chen et al. [9] reported that dietary supplementation with 1% palygorskite enhanced intestinal T-SOD activity in broilers. Clay minerals can improve antioxidant function, possibly by adsorbing mycotoxins, heavy metals, and pathogenic microorganisms [5-9], thereby reducing contact between harmful substances and intestinal mucosa and alleviating tissue oxidative stress [36]. The present study found that dietary supplementation with ultra-fine grinding

palygorskite significantly increased GSH-Px activity in broiler breast muscle, whereas regular grinding palygorskite supplementation did not, indicating that ultra-fine grinding palygorskite can enhance the antioxidant capacity of breast muscle. This may be because ultra-fine grinding palygorskite, with its larger specific surface area, can better adsorb harmful substances and reduce oxidative stress caused by these substances. Additionally, studies have shown that the regulation of antioxidant capacity by palygorskite is related to its surface active groups [37]. Therefore, the superior effect of ultra-fine grinding palygorskite on improving muscle antioxidant capacity may also be attributed to the mechanical activation of palygorskite surface during the ultra-fine grinding process, which generates new ionic bonds or active sites [38].

4 Conclusion

Dietary supplementation with ultra-fine grinding palygorskite in broilers can reduce heavy metal residues in muscle, affect muscle color, and enhance the antioxidant capacity of breast muscle, demonstrating superior effects compared with regular grinding palygorskite at the same inclusion level.

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Note: Figure translations are in progress. See original paper for figures.