Effects of Dietary Supplementation of Superoxide Dismutase Mimetics on Muscle Fiber Characteristics and Muscle Superoxide Dismutase Activity in Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with superoxide dismutase mimetic (SODm) on muscle fiber characteristics and muscle superoxide dismutase (SOD) activity in broiler chickens. A total of 360 one-day-old Arbor Acres (AA) broiler chickens were selected and randomly divided into 6 groups, with 6 replicates per group and 10 chickens per replicate. Each group was fed experimental diets with SODm levels of 0 (control), 1.0‰, 1.5‰, 2.0‰, 2.5‰, and 3.0‰, respectively, and the experimental period lasted for 42 days. The results showed that there were no significant differences in growth performance indices among the groups ($P>0.05$). The breast muscle fiber density of broilers in the 1.0‰ SODm group was significantly higher than that in the other groups ($P<0.05$), and the breast muscle fiber diameter of broilers in the 1.5‰ SODm group was significantly higher than that in the other groups except the 2.0‰ SODm group ($P<0.05$). The leg muscle fiber density of broilers in the 1.5‰ SODm group was significantly higher than that in the other groups ($P<0.05$), and the leg muscle fiber diameter of broilers in the 1.5‰ SODm group was significantly lower than that in the other groups ($P<0.05$). The breast muscle shear force of broilers in the 2.0‰ SODm group was significantly lower than that of the control group ($P<0.05$). The breast muscle SOD activity of broilers in the 3.0‰ SODm group was significantly higher than that in the other groups ($P<0.05$), and the leg muscle SOD activity of broilers in the 1.5‰ SODm group was significantly higher than that in the other groups except the 3.0‰ SODm group ($P<0.05$). It can be concluded that dietary supplementation with SODm can improve muscle fiber characteristics, increase SOD activity in muscle, improve meat quality to a certain extent, and simultaneously enhance the antioxidant capacity of muscle in broiler chickens.

Full Text

Effects of Dietary Superoxide Dismutase Simulant on Myofiber Characteristics and Muscle Superoxide Dismutase Activity in Broilers

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Abstract

This study investigated the effects of dietary superoxide dismutase simulant (SODm) on myofiber characteristics and muscle superoxide dismutase (SOD) activity in broilers. A total of 360 one-day-old Arbor Acres (AA) broiler chickens were randomly allocated into 6 groups with 6 replicates per group and 10 birds per replicate. The groups were fed experimental diets containing 0 (control), 1.0‰, 1.5‰, 2.0‰, 2.5‰, or 3.0‰ SODm for a 42-day experimental period. The results showed no significant differences in growth performance indices among all groups ($P>0.05$). The breast muscle fiber density in the 1.0‰ SODm group was significantly higher than in other groups ($P<0.05$), while the breast muscle fiber diameter in the 1.5‰ SODm group was significantly greater than in all groups except the 2.0‰ SODm group ($P<0.05$). The leg muscle fiber density in the 1.5‰ SODm group was significantly higher than in other groups ($P<0.05$), and the leg muscle fiber diameter in this group was significantly lower than in all other groups ($P<0.05$). The breast muscle shear force in the 2.0‰ SODm group was significantly lower than in the control group ($P<0.05$). The breast muscle SOD activity in the 3.0‰ SODm group was significantly higher than in all other groups ($P<0.05$), while the leg muscle SOD activity in the 1.5‰ SODm group was significantly higher than in all groups except the 3.0‰ SODm group ($P<0.05$). In conclusion, dietary SODm supplementation can improve myofiber characteristics, enhance muscle SOD activity, improve meat quality to a certain extent, and strengthen muscle antioxidant capacity in broilers.

Keywords: superoxide dismutase simulant; broilers; muscle; myofiber; antioxidant

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Superoxide dismutase simulant (SODm) is a synthetic compound designed to mimic the function of superoxide dismutase (SOD). In addition to possessing SOD' s anti-aging, stress-resistance, antioxidant, and immunity-enhancing properties [1], SODm overcomes the limitations of natural SOD, including its large molecular weight, poor thermal stability, and susceptibility to inactivation. SOD is primarily classified into three types: iron superoxide dismutase (Fe-SOD), copper-zinc superoxide dismutase (Cu/Zn-SOD), and manganese su-

peroxide dismutase (Mn-SOD) [2]. SODm is mainly synthesized based on the structure of Mn-SOD. The application of SODm in plants has been promoted to some extent, demonstrating its ability to increase sugar content in sugar beets while improving their growth status [3], and to enhance yields in corn and cotton [4-6]. These agricultural applications have primarily highlighted SODm' s stress-resistance properties. If SODm' s antioxidant and anti-aging characteristics could be applied to livestock and poultry to improve muscle quality and antioxidant capacity, it would further advance the animal husbandry industry.

Myofibers are essential components of muscle and represent a critical indicator of meat quality. Therefore, studying myofiber density and diameter is highly significant for evaluating meat quality. Maltin et al. [7] reported that in poultry meat quality research, myofibers serve as one of the key indicators for measuring muscle tenderness. Additionally, Chen et al. [8] found that larger muscle fiber values correlate with higher inosinic acid content and better meat quality, while increased myofiber density corresponds to smaller fiber diameters [9]. Studies on exogenous antioxidants have shown that dietary supplementation with 0.01% L-carnosine can increase SOD and glutathione peroxidase (GSH-Px) expression in finishing pigs [10], and that catechins can enhance polyunsaturated fatty acid content and extend shelf life in goat meat [11-12]. Whether SODm, as an exogenous antioxidant, can achieve similar antioxidant effects in poultry production warrants investigation.

Therefore, this study examined the effects of different dietary SODm levels on growth performance, myofiber characteristics, and muscle SOD activity in broilers to provide a theoretical basis for SODm application in poultry diets.

Materials and Methods

Experimental Animals and Design

SODm was provided by Daqing High-Tech Zone Huamei Technology Co., Ltd. A total of 360 one-day-old Arbor Acres (AA) male broiler chicks were randomly divided into 6 groups with 6 replicates per group and 10 birds per replicate. The experiment lasted from 1 to 42 days of age. A single-factor experimental design was employed, as detailed in Table 1 .

Basal Diet and Management

The corn-soybean meal basal diets were formulated according to NRC (1994) nutrient requirements for broilers and the *Feed Composition and Nutritional Value Tables of China* (26th edition, 2015). The composition and nutrient levels of the basal diets are presented in Table 2 . Birds were housed in three-tier cage systems and raised according to standard broiler management practices.

The premix provided the following per kilogram of diet: for days 1-21, 1.5 g of 50% choline chloride, 12,000 IU VA, 3,000 IU VD, 24 IU VE, 2.5 mg VK3, 2.2 mg thiamine, 11 mg pantothenic acid, 35 mg niacin, 0.18 mg biotin, 0.6 mg folic

acid, 3.8 mg pyridoxine, 0.011 mg VB12, 100 mg Fe (as ferrous sulfate), 120 mg Mn (as manganese sulfate), 100 mg Zn (as zinc sulfate), 8 mg Cu (as copper sulfate), 0.7 mg I (as potassium iodide), and 0.3 mg Se (as sodium selenite). For days 22-42, the premix composition was identical. All nutrient levels were calculated values.

Sample Collection and Processing

Daily feed intake was recorded for each group throughout the experiment to calculate average daily feed intake. Initial and final body weights were recorded to determine average daily gain and feed-to-gain ratio.

At 6 weeks of age, two healthy broilers approaching the average body weight were selected from each replicate, slaughtered by neck bleeding, and the left breast and leg muscles were dissected. For histological analysis, samples measuring 1.5 cm × 0.6 cm × 0.6 cm were excised along the muscle fiber orientation, immediately fixed in formalin solution, and processed according to standard paraffin embedding procedures. The samples were dehydrated in 70%, 80%, 90%, 100% (first), and 100% (second) ethanol for 1 hour each, cleared in xylene (first for 15 minutes, second for 10 minutes), infiltrated with melted paraffin (first, second, and third baths) for 1 hour each in a thermostat, and finally embedded. Sections were cut at 6 μm thickness using a Leica 2135 microtome, stained with hematoxylin-eosin, and observed under a Moticam-3000 microscopic imaging system.

For SOD activity analysis, 0.5 g of fresh breast and leg muscle samples were collected from two birds per replicate, washed in physiological saline, placed in 10 mL EP tubes with 4.5 mL of ice-cold 0.9% physiological saline, and homogenized (10 seconds per cycle, 10-second intervals, 5 cycles) to prepare 10% homogenates, which were stored at -20 °C.

For meat tenderness determination, left breast and leg muscles were collected from two birds per replicate, sealed in self-sealing bags, and stored at 4 °C.

Measurement Methods

Myofiber diameter was measured at 400× magnification. Three fields were selected per sample, 20 myofibers per field were photographed, and their cross-sectional areas were measured using Image-Pro Plus 6.0 pathology image analysis software, with averages calculated.

Myofiber density was determined at 400× magnification by selecting three fields per sample, measuring the total area of each field using Image-Pro Plus 6.0, and counting the number of myofibers (N) across the three fields to calculate fiber density.

Muscle SOD activity was measured using a water-soluble tetrazolium salt (WST-1) SOD activity assay kit according to the manufacturer' s instructions.

Meat tenderness was assessed by sealing breast and leg muscles in self-sealing bags, immersing them in an 80 °C water bath for 0.5 hours, cooling in 15 °C running water for 40 minutes, blotting surface moisture with filter paper, and measuring shear force using a C-LM3B digital muscle tenderness meter (developed by Northeast Agricultural University Engineering College). Each sample was sheared three times and the average value was recorded.

Statistical Analysis

Data were organized using Excel 2016 and analyzed using SAS 9.4 software. One-way ANOVA and Duncan' s multiple comparison tests were performed. Results are expressed as "mean ± standard deviation," with $P<0.05$ considered statistically significant. Cubic curve regression equations were fitted using Excel 2016.

Results

Effects of SODm on Broiler Growth Performance

As shown in Table 3 , dietary supplementation with different SODm levels had no significant effect on broiler growth performance indices ($P>0.05$).

Effects of SODm on Myofiber Characteristics

Breast Muscle Table 4 shows that the breast muscle fiber density in the 1.0‰ SODm group was significantly higher than in all other groups ($P<0.05$), while the control group exhibited intermediate values. The breast muscle fiber diameter was largest in the 1.5‰ SODm group, significantly greater than in all groups except the 2.0‰ SODm group ($P<0.05$). The 1.0‰ SODm group had the smallest fiber diameter, significantly lower than all other groups ($P<0.05$), with the control group showing intermediate values. Histological sections (Figure 1 [Figure 1: see original paper]) visually confirmed these effects, showing higher fiber density in the 1.0‰ SODm group and larger fiber diameter in the 1.5‰ SODm group.

Leg Muscle As presented in Table 5 , the leg muscle fiber density in the 1.5‰ SODm group was significantly higher than in all other groups ($P<0.05$), while the 1.0‰ SODm group showed intermediate values. The leg muscle fiber diameter was smallest in the 1.5‰ SODm group, significantly lower than in all other groups ($P<0.05$). The 3.0‰ SODm group exhibited the largest fiber diameter, with the control group showing slightly lower but non-significant values ($P>0.05$). Figure 2 [Figure 2: see original paper] illustrates these effects, demonstrating higher fiber density and smaller fiber diameter in the 1.5‰ SODm group.

Effects of SODm on Muscle Tenderness

Table 6 indicates that the breast muscle shear force was lowest in the 2.0‰ SODm group, significantly lower than in the control group ($P<0.05$), with no significant differences among other groups ($P>0.05$). The leg muscle shear force in the 1.5‰ SODm group was slightly lower than in the control group, but the difference was not significant ($P>0.05$).

Effects of SODm on Muscle SOD Activity

According to Table 7 , the breast muscle SOD activity was highest in the 3.0‰ SODm group, significantly higher than in all other groups ($P<0.05$). The 2.5‰ and 1.0‰ SODm groups showed intermediate values that did not differ significantly from the control group ($P>0.05$), while the control group exhibited the lowest activity. The leg muscle SOD activity was highest in the 1.5‰ SODm group, significantly higher than in all groups except the 3.0‰ SODm group ($P<0.05$).

Optimal SODm Supplementation Level

Based on the above results, one-way ANOVA revealed that dietary SODm supplementation significantly affected breast and leg muscle fiber diameter and density, muscle tenderness, and muscle SOD activity ($P<0.05$), though it had no significant impact on growth performance ($P>0.05$). Curve fitting analysis was therefore performed.

Table 8 presents the cubic regression equations and calculated optimal SODm levels. For breast muscle fiber characteristics, the suitable dietary SODm level ranged from 0.4‰ to 1.8‰. Notably, breast muscle SOD activity showed an initial increase followed by a decrease when SODm levels were between 0 and 1.5‰, peaking at 0.9‰ SODm, then increasing again at levels from 1.5‰ to 3.0‰. For leg muscle fiber characteristics, the optimal SODm level was 0.9‰ to 1.6‰, with leg muscle SOD activity following the same trend as breast muscle. Overall, the dietary SODm level that significantly improved myofiber characteristics in broilers ranged from 0.4‰ to 1.8‰.

Discussion

Effects of SODm on Broiler Growth Performance

Recent studies have investigated the application of exogenous antioxidants in animal production. Hamano et al. [13] found that lipoic acid effectively reduced plasma triglyceride content but had minimal effect on average daily gain and feed-to-gain ratio in broilers [14]. Zou et al. [15] reported that dietary antioxidant supplementation increased serum SOD activity in broilers without significantly affecting production performance. The current study' s finding that SODm supplementation did not significantly affect broiler growth performance aligns with these previous results. This may be because SODm primarily

functions to enhance immunity and anti-aging effects, and its nearly odorless nature does not significantly improve diet palatability, resulting in relatively minor effects on growth performance.

Effects of SODm on Broiler Meat Quality

Antioxidants can improve poultry meat quality, which is largely reflected in myofiber characteristics. Smaller myofiber diameter and higher density are associated with better meat quality [16-18]. Myofibers are classified into four types: slow oxidative (Type I), fast oxidative (Type IIa), fast glycolytic (Type IIb), and intermediate (Type IIx) [19-20]. Type I fibers have smaller diameters, lower shear force, and better tenderness characteristics [21]. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1) can induce mitochondrial synthesis [22] and expression of Type I myofiber-specific genes, effectively converting fast-twitch to slow-twitch fibers [23]. Peroxisome proliferator-activated receptor delta (PPAR), an upstream regulator of PGC-1 , can promote PGC-1 expression and induce slow-twitch fiber formation [24]. Exercise can increase PGC-1 and PPAR responses, promoting myofiber conversion [25]. As SODm possesses antioxidant and immunity-enhancing properties, maintaining healthy physiological status may relatively increase physical activity. This study confirmed that SODm effectively reduced myofiber diameter, increased fiber density, and improved muscle tenderness. Liu et al. [26] found that myofiber diameter positively correlated with muscle tenderness, while fiber density showed an inverse relationship. Han et al. [27] also reported that myofiber density was significantly positively correlated with meat quality, with higher density associated with more tender meat. The current results demonstrate an inverse relationship between fiber diameter and density, and a positive correlation between fiber diameter and muscle tenderness.

Effects of SODm on Muscle SOD Activity

As an exogenous antioxidant, SODm can enhance muscle total antioxidant capacity (T-AOC) and SOD activity, strengthening the antioxidant capacity of post-slaughter muscle [28]. High-level methionine supplementation can increase breast muscle T-AOC [29]. SOD is a primary antioxidant enzyme, and its activity represents the body' s ability to scavenge free radicals [30] and maintain normal cell growth [31]. In this study, muscle SOD activity initially increased, then decreased, and subsequently increased again with rising dietary SODm levels, ultimately improving muscle antioxidant capacity.

Notably, SODm effects on breast and leg muscle quality were not synchronized in this study, as the same supplementation level could not simultaneously improve myofiber characteristics in both muscle types. Since SODm application in animals remains limited, the reasons for this asynchronous effect require further investigation.

Conclusion

Dietary SODm supplementation had no significant effect on broiler growth performance but improved muscle tenderness, myofiber characteristics, and muscle SOD activity. Dietary SODm levels of 0.4‰ to 1.8‰ effectively increased breast and leg muscle fiber density, reduced fiber diameter, improved muscle tenderness, and enhanced muscle SOD activity.

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