

Effects of dietary supplementation with selenium yeast and jujube powder on mitochondrial oxidative damage and apoptosis of chicken

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ABSTRACT The main objective of this study was to explore the effects of dietary selenium yeast and jujube powder on mitochondrial oxidative damage and cell apoptosis of broilers during postmortem aging, chicken breasts of broilers fed diets supplemented with different concentrations of selenium yeast and jujube powder were used as research subjects. With the prolongation of postmortem aging time, the levels of reactive oxygen species (ROS), carbonyl content, mitochondrial permeability transition pore (MPTP) openness, and mitochondrial membrane permeability increased significantly ($P < 0.05$). The contents of the sulfhydryl, mitochondrial membrane potential, shear force, and cytochrome C (Cyt-c) reduction level decreased significantly ($P < 0.05$). The activity of Caspase-3 and Caspase-9 increased from 0 to 24 h postmortem but fell from 24 to 72 h postmortem. Compared with the control group, dietary selenium yeast and jujube

powder significantly reduced mitochondrial oxidative damage. They greatly increased the shear force, mitochondrial membrane potential, and Cyt-c reduction levels ($P < 0.05$). Among them, the combination group of high-dose selenium yeast and jujube powder had more significant effects on ROS scavenging, reducing cell membrane permeability, protecting cell membrane integrity, and increasing Cyt-c reduction level ($P < 0.05$). In conclusion, cell apoptosis intensifies during the chicken breast's aging time, and muscle tenderness continues. Still, different doses of dietary selenium yeast and jujube powder can inhibit mitochondrial oxidation to various degrees. The combined group of selenium yeast and jujube powder with $0.6 \text{ mg}\cdot\text{kg}^{-1}$ has the best effect. This study is of great significance for applying natural antioxidant ingredients such as selenium yeast and jujube powder in the development and utilization of poultry feed.

Key words: chicken, selenium yeast, jujube powder, apoptosis, tenderness

2022 Poultry Science 101:102072
<https://doi.org/10.1016/j.psj.2022.102072>

INTRODUCTION

Apoptosis is a process that livestock cells must undergo and a programmed death process after slaughter. Caspases, an IL-1 β converting protease, are involved in apoptosis and have an essential impact on meat tenderness (Kopeina et al., 2018). To maintain the balance of the internal environment, body cells will undergo rapid apoptosis due to severe stress changes, such as ischemia, hypoxia, and gradual cell exhaustion (Sies et al., 2017; Liu et al., 2021). As the supply of nutrients and oxygen to the cells is interrupted after slaughter and bloodletting (Stojnev et al., 2013), reactive oxygen species accumulated by cellular peroxidation can regulate mitochondrial permeability and transport pores, causing

mitochondrial oxidative damage until cellular dysfunction and even apoptosis. After the animal is slaughtered, apoptosis begins to occur. As one of the main apoptosis pathways, mitochondrial apoptosis is mainly involved in activating Caspase-3 (Twiddy et al., 2004; Guo et al., 2021). With the prolongation of aging time, the accumulation of intracellular reactive oxygen species, and the weakening of the body's antioxidant capacity, mitochondria are the prominent organelles that produce reactive oxygen species and are susceptible to oxidative damage. When mitochondria undergo structural changes after slaughter, proapoptotic factors are released from damaged mitochondria, activating apoptotic enzymes and participating in postmortem muscle tenderization (Chen et al., 2011; Eleftheriadis et al., 2016; He et al., 2018; Hood et al., 2019; Kemp et al., 2010). It was found that treatment of postmortem yak meat with N, N, N, 'N'-tetramethylphenylenediamine dihydrochloride attenuated mitochondrial oxidative damage and significantly inhibited the activities of Caspase-3 and Caspase-9, resulting in decreased tenderness (Wang et al., 2018). In addition, Caspase-3 inhibitors inhibited mitochondrial oxidative

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Received May 6, 2022.

Accepted July 20, 2022.

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damage, reduced the release of cytochrome c from mitochondria to the cytoplasm, reduced the activity of Caspase-3/9, and ultimately affected myofibrillar protein degradation (Li et al., 2022). Therefore, oxidative damage to mitochondria during postmortem aging can affect the tenderization process of muscle by regulating apoptosis.

Factors such as feeding before slaughter, transportation, and refrigeration after slaughter potentially affect the apoptosis of muscle cells after slaughter (Bortoluzzi et al., 2016; Katiy et al., 2020), and feeding before slaughter can increase the activity of antioxidant enzymes in muscle by regulating the content of trace elements and nutrients in muscle mass, thereby affecting meat quality (Heng et al., 2021). Numerous studies have shown that dietary supplementation of natural or synthetic antioxidants could significantly improve the antioxidant properties of postmortem muscles and simultaneously have a specific effect on apoptosis. Chen et al. found that dietary supplementation of selenium yeast could dramatically reduce oxidative stress induced by cadmium, suggesting that dietary supplementation of selenium yeast could dramatically reduce oxidative stress in the kidneys of broilers and then down-regulate the expression of apoptotic genes (Chen et al., 2021). Lu et al. found that supplementing taurine in broiler basal diets significantly reduced mitochondrial oxidative damage in postmortem chicken breast (Lu et al., 2019). Moreover, dietary selenium yeast supplementation significantly improved the activity of glutathione peroxidase (GSH-Px) in the serum of Tibetan sheep after slaughter, suggesting that dietary selenium yeast supplementation can provide enough selenium to synthesize GSH-Px, thereby improving the activity of GSH-Px in cells (Wang et al., 2019). In addition to adding trace elements to the diet, red dates can be added to the daily diet of animals as a plant-derived natural substance rich in active antioxidant substances. It was found that 3 polysaccharide components, ZP1, ZP2, and ZP3 from jujube fruit, and ZP3 had a significant scavenging effect on hydroxyl free radicals, suggesting that galacturonic acid in the polysaccharide component had a scavenging effect on free radicals (Liu et al., 2020). These results indicate that jujube can act as a natural antioxidant by scavenging reactive oxygen species, reducing the level of oxidative stress in mitochondria and thereby delaying apoptosis.

It is common to add natural antioxidants such as selenium yeast and jujube powder to the diet, which can significantly reduce the level of oxidative stress in postmortem animals, thereby improving meat quality. However, it has not been reported whether mitochondrial oxidative damage and apoptosis can be reduced by improving diet, affecting chicken tenderness. Therefore, the selenium content, antioxidant enzyme activity, oxidative damage degree, apoptosis enzyme activity, and chicken myofibrils were measured in this study. The effects of selenium yeast and jujube powder on oxidative damage and apoptosis of chicken mitochondria and tenderness of chicken were studied, which provided a

theoretical basis for nutritional regulation of maturity and tenderness of chicken after slaughter.

MATERIALS AND METHODS

Animals and Muscle Sampling

A total of 360 one-day-old white-feathered male chickens were divided into 6 groups, with 6 parallel chickens in each group and 10 chickens in each parallel group. A basal diet (corn-miscellaneous meal type) was used to prepare powdery compound diets according to the nutritional requirements of NRC (1994). The diets were divided into 6 groups according to the supplemental jujube meal and selenium yeast levels. They were CK (basal diet), J (basal diet +8% jujube meal replacing with 8% corn), 0.3S group (basal diet +0.3 mg·kg⁻¹ selenium yeast), 0.6S group (basal diet +0.6 mg·kg⁻¹ selenium yeast) and 0.3S+J (basal diet +0.3 mg·kg⁻¹ selenium yeast +8% jujube meal replacing 8% corn) and 0.6S+J (basal diet +0.6 mg·kg⁻¹ selenium yeast +8% jujube meal replacing 8% corn) groups. Under the same feeding conditions, the feeding period lasted for 42 d, divided into 2 stages (0–21 d) and (22–24 d). The basal diet levels and nutritional levels are shown in Table 1. After feeding, 360 white-feathered broilers were cut off the jugular veins and bled for 2 min. The breast was cut off, and made every effort to ensure the organization's integrity. The shear force was quickly determined for 0 h data, while the sample was placed at -80°C. Meat samples were aged at 4°C for 12, 24, 48, and 72 h, respectively. After each aging period, take 3 g meat sample to measure shear force, and the remaining samples were stored in -80°C liquid nitrogen to determine other indicators. All animal handling protocols were approved by the Animal Care and Use Committee of the Poultry Institute.

Reagents

DNPH, PIPES, DTNB, EDTA, NaN₃, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, guanidine hydrochloride, hydrochloric acid, potassium chloride, Triton-X-100 Tianjin Guangfu Technology Development Co., LTD.; NaCl, CuSO₄·5H₂O, NaOH and Na₃PO₄ were analytical pure, purchased from Sino-pharmaceutical Group Co., Ltd. The test kits were all used by Beijing Solebo Bioengineering Institute.

Selenium Content

The Se concentration of each muscle sample was determined according to the method described by Mousaie et al. (2017) and slightly modified. The selenium content was determined by atomic fluorescence spectrometry: the sample was accurately weighed 1 g, and 12 mL HNO₃/H₂O₂ (3:1) solution was added and mineralized in a microwave oven. Then add 1.9 mL of 8 mol/L hydrochloric acids and heat the sample with a microwave at 650 W for 6 min. The selenium (VI) was directly

Table 1. Basic diet composition and nutritional level.

Item	Early stage (0–21 d)		Late (22–42 d)	
	Corn type	Date powder type	Corn type	Date powder type
Diet composition				
Corn%	64.16	53.36	65.27	54.75
Date powder%	0.00	6.00	0.00	8.00
Vegetable oil%	2.00	3.60	3.50	4.80
Soybean meal%	20.00	21.20	16.00	17.20
Cottonseed meal%	3.00	3.00	4.00	4.00
Corn protein flour%	3.00	3.00	3.00	3.00
Rapeseed meal%	3.00	3.00	4.00	4.00
Stone powder%	1.20	1.20	1.10	1.10
CaHPO ₄ %	1.70	1.70	1.20	1.20
Premix%	0.50	0.50	0.50	0.50
Salt%	0.40	0.40	0.35	0.35
Methionine%	0.15	0.15	0.16	0.16
Lysine%	0.50	0.50	0.48	0.48
NaHCO ₃ %	0.20	0.20	0.24	0.24
Choline chloride%	0.20	0.20	0.20	0.20
Nutritional level				
Metabolizable energy MJ/kg	12.34	12.34	12.97	12.97
Crude protein%	20.00	20.00	18.00	18.00
Ca%	1.00	1.00	0.80	0.80
Available phosphorus%	0.45	0.45	0.35	0.35
Lysine%	1.15	1.15	1.05	1.05
Methionine%	0.54	0.54	0.40	0.40

Note: Premix provides 15000 IU of vitamin A, 5100 IU of vitamin D3, 25.5 mg of vitamin E, 2.5 mg of vitamin K, 3.6 mg of vitamin B1, 6.9 mg of vitamin B2, 4.5 mg of vitamin B6, 0.036 mg of Vitamin B12, 66.3 mg of niacin, 18 mg of calcium pantothenate, 0.18 mg of biotin, 1.4 mg of folic acid, 55 mg of Fe, 8 mg of Cu, 60 mg of Zn, 60 mg of Mn, 0.2 mg of Se, 0.3 mg of I. In addition, metabolizable energy is calculated according to the composition of raw materials (based on dry matter), and other values are measured.

digested into selenium (IV). Then dilute the sample with 4 mol/L hydrochloric acids until the volume reached 10 mL. Then atomic fluorescence spectrometer (AFS-200S111; Jiangsu Skyray Instrument Co., Ltd., Kunshan, China) was used to determine.

Extraction of Myofibrin

Myofibers were extracted according to previously reported methods with slight modifications (Wang et al., 2020). Meat muscle was cut into small pieces, and at 4°C with phosphate buffer (0.02 mmol/L, pH 7.0) will be chopped meat samples homogenization (Liu et al., 2021), then use a frozen centrifuge (TGL-16MC; Hunan Xiangyi Group, Xiangyi, China) at 4°C at 8,000 r/min for 20 min to isolate the sarcoplasm. To separate sarcoplasmic protein. The resulting particles were washed twice with phosphate buffer solution (0.02 mmol/L PB, pH 7, and 0.6 mol/L KCl) and centrifuged as described above. After the second washing, the supernatant was filtered with filter paper, and the filtrate was myofibrin. Myofibrin was stored at -80°C.

Carbonyl Content

The measurement method was done according to a previous method, with some changes (Zarei et al., 2019). The concentration of myofibrin was adjusted to 5 mg·mL⁻¹ with 100 mmol·L⁻¹ KCl solution, and 0.5 mL myofibrin solution was added to 2 mL 2 mol·L⁻¹ HCl solution (control) and 2 mL solution containing 0.2% dinitrophenylhydrazine 2 mol·L⁻¹ HCl, respectively. The precipitated protein was added with 2 mL 20%

trichloroacetic acid and centrifuged at 10,000 r·min⁻¹ at 4°C for 5 min. The precipitation was cleaned 3 times with 2 mL [ethyl acetate:ethanol (1:1)]. After the reagents in the sediment were volatilized, 3 mL 6 mol·L⁻¹ guanidine hydrochloride was added and placed in 37°C water bath for 30 min. Then the reaction solution was centrifuged for 5 min at 4°C and 10,000 r·min⁻¹. The absorbance of the supernatant was measured at 370 nm wavelength with the protein extract as a blank control (SP-756P; Shanghai Meipuda Instrument Co., LTD, Shanghai, China). The cuvette is 1 cm thick. The carbonyl content was calculated using the molecular absorbance coefficient of 22,000 m⁻¹·cm⁻¹.

Sulfhydryl Content

According to the method of Liu et al. (2000), it was slightly modified. In 0.5 mL 2 mg·mL⁻¹ protein solution, 2 mL sodium dodecyl sulfate solution, and 0.5 mL 10 mmol·L⁻¹ 2-nitrobenzoic acid reagent were added successively, and the reaction was conducted at room temperature for 15 min. The absorbance value A was measured at 412 nm (SP-756P; Shanghai Meipuda Instrument Co., Ltd, Shanghai, China). 0.5 mL 25 mmol·L⁻¹ potassium phosphate buffer (pH 6.25) replaced protein solution as blank control. The total sulfhydryl content was calculated using the molecular absorbance coefficient of 13,600 m⁻¹·cm⁻¹.

Isolation of Mitochondria

Mitochondria were isolated as described previously with slight modification (Wang et al., 2018). Take 2 g of

chicken breast, minced muscles were homogenized with buffer A (containing 70 mM sucrose, 220 mM mannitol, 2.0 mM ethylene diamine tetra-acetic acid, 5.0 mM 4-morpholinepropanesulphonic acid, and 0.5% bovine serum albumin [BSA]; pH 7.4) (weight: volume = 1/10). After centrifugation of these homogenates (1,000 g, 10 min, 4°C), the supernatants were again centrifuged at 1,000 g for 10 min at 4°C. Subsequently, the supernatant was centrifuged at 8,000 g for 20 min at 4°C with buffer B (obtained by diluting 7.5-fold buffer A without BSA, pH 7.4) to obtain the mitochondria and cytosol. The resulting supernatant contained cytosolic-enriched protein, and the resultant mitochondrial pellets were suspended in buffer B. The protein concentration was determined using the biuret method.

Reactive Oxygen Species Content

ROS determination was analyzed according to the method by Zhu et al. with several modifications (Zhu et al., 2017). Placing 1 g of muscle sample in 4 times the volume of precooled buffer solution (10 mmol/L Tris-HCl, 10 mmol/L sucrose, 0.1 mmol/L EDTA-2Na, 0.8% [W/V] NaCl, pH 7.4). Homogenate was centrifuged at 3,000 g at 4°C for 15 min. The supernatant was rapidly mixed with reaction buffer (10 mmol/L Tris-HCl, 10 mmol/L sucrose, 0.1 mmol/L EDTA-2Na, 0.8% [W/V] NaCl, 10 μ mol/L DCFH-Da, pH 7.4) in a 1:1 ratio. After incubation at 37°C for 30 min, fluorescence intensity was measured at an excitation wavelength of 488 nm and 525 nm by a fluorescence spectrophotometer (RF-5301PC; Shimadzu Corporation of Japan, Shanghai, China).

Mitochondrial Membrane Potential

The mitochondrial membrane potential assay kit (Beyotime, Beijing, China) was used according to the manufacturer's protocol. 0.1 mL purified mitochondrial pellets (100 μ g/mL of protein) were incubated with 0.9 mL JC-1 dyeing working solution for 20 min, and the fluorescence intensity was immediately measured using a fluorescence spectrophotometer (Shimadzu RF 5301, Kyoto, Japan). The wavelengths for detecting the monomeric and aggregated forms of JC-1 were 490/530 and 525/590 nm (excitation/emission).

Mitochondrial Membrane Permeability

Muscle samples were added with 100 mL of separation solution (250 mmol/L sucrose, 10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.4), of which 10 g of meat sample was required; after homogenating at 10,000 g for 2 min, centrifuged at 1,500 g at 4°C for 15 min. The supernatant was centrifuged at 12,000 g at 4°C for 20 min. The precipitation was washed twice with the extraction solution and mixed with buffer solution (250 mmol/L sucrose, 10 mmol/L Tris-HCl, pH 7.4) to obtain a mitochondrial suspension. The concentration of

mitochondrial protein was determined by the biuret method. Quickly add 300 μ L (3 g/L) of the diluted mitochondrial solution to a 2,700 μ L test medium (230 mmol/L mannitol, 70 mmol/L sucrose, 3 mmol/L HEPES, PH 7.4). After incubation at 25°C for 3 min, the absorbance value was measured at 540 nm. 3 mL test medium as the control, the test process to ensure the same sample loading.

Mitochondrial Permeability Transition Pore Opening

Mitochondria were extracted by the previous method, and the concentration was determined by the biuret method. The concentration was adjusted to 0.3 mg/mL with the test medium of mitochondrial permeability conversion hole, and the absorbance value was measured at 540 nm. The greater the absorbance, the smaller the opening degree of the hole.

Cytochrome C Reduction Levels

According to the method of Borutaite and Brown (2007), the cytoplasmic suspension was prepared. A certain amount of cytoplasmic suspension was taken, and the absorbance was measured at 550 and 535 nm, respectively. The reduction level of cytoplasmic Cyt-c was expressed by the ratio of the difference of absorbance values at different wavelengths to protein concentration.

Caspase-9 and Caspase-3 Activities

According to the method of Chen et al. (2011), 160 mg of meat sample was taken, cut into pieces, and 1 mL of Caspase-3/9 lysate was added and homogenized 30 times with a glass homogenate in an ice water bath. The homogenate was centrifuged at 4°C for 10 min (10,000 g), the supernatant was taken for measurement, and the protein concentration was determined by the Bradford method. 170 μ L Caspase-3/9 buffer, 20 μ L supernatant to be measured (protein stock), 10 μ L DEVD-pNA, 2 mmol·L⁻¹ Caspase-3/9 substrates were successively added to 96-well ELISA plate, 190 μ L reaction buffer was added to the control well. At 37°C for 2 h, the absorbance at 405 nm was measured with a microplate reader.

Glutathione Peroxidase and Total Antioxidant Capacity Activities

Chicken breast samples were homogenized in phosphate buffered saline (PBS) ice bath, and the meat sample weight was 1 g. Centrifuge at 8,000 g for 10 min at 4°C and take the supernatant for testing. The determination was carried out according to the Beijing Soleibao Bioengineering Institute kit.

Myofibrin Fragmentation Index

The measurement of myofibrin fragmentation index (MFI) was slightly modified by referring to the method of Wang et al. (2018). Take 2 g of dressed chicken, ground it, and add 20 mL MFI buffer (100 mmol·L⁻¹ KCl, 8.8 mmol·L⁻¹ KH₂PO₄, 1 mmol·L⁻¹ EGTA, 11.2 mmol·L⁻¹ K₂HPO₄, 1 mmol·L⁻¹ MgCl₂, 1 mmol·L⁻¹ NaN₃), homogenized 3 times at high speed, 20 s each time (1 min interval), centrifuged at 1000 g for 15 min. Discard the supernatant, add 20 mL buffer solution to the precipitation, stir well, centrifugal once; The precipitate was added into 5 mL buffer solution, then mixed evenly, and the connective tissue was filtered out. Wash the filter residue with 5 mL buffer. The filtrate protein was prepared into (0.5 ± 0.05) mg·mL⁻¹ with MFI buffer, and the optical density value was measured at 540 nm.

Shear Force of Chicken Breast

The determination of shear force referred to Chen et al., 2017b method and is slightly modified. The chicken breast meat was steamed and cooled, and the meat samples were sliced according to the cross-section specification of 1 cm × 1 cm. Then, the tenderness instrument was used to cut the muscle fibers of the meat pieces vertically (C-LM4; Harbin, China), and the 3 groups of data were recorded and averaged.

Statistical Analysis

Excel 2019 was used to calculate the mean value as the test data, SPSS 20.0 was used to conduct one-way ANOVA and two-factor interaction analysis for the data, Origin 2021 was used to draw the principal component analysis biplot, and correlation heat map and Duncan's new complex range method was used for significance analysis ($P < 0.05$).

RESULTS

Selenium Content in Postmortem Chicken Breast

The changes of selenium content in the chicken breast under different selenium yeast and jujube powder

treatments are shown in Table 2. At 0 h after slaughter, the addition of selenium yeast (organic selenium) and jujube powder could significantly increase the content of selenium in chicken after slaughter compared with the control group ($P < 0.05$). Compared with the control group, the selenium content of chicken breast meat in 0.3S and 0.6S groups was increased by 96% and 156%, respectively, while that in 0.3S+J and 0.6S+J groups was increased by 124% and 216%, respectively, and the selenium content in the 0.6S+J group was significantly higher than that in other feeding groups ($P < 0.05$), and the selenium content reached 0.79 mg·kg⁻¹. It can be seen that the addition of selenium yeast in the diet can effectively enrich the content of selenium in chicken meat through metabolism and accumulation, and there is a dose-time effect relationship ($P < 0.01$).

Reactive Oxygen Species Content in Postmortem Chicken Breast

It can be seen from Table 3 that the ROS content in chicken breast gradually increased with postslaughter maturity. Still, adding selenium yeast and jujube powder to the diet could significantly reduce the ROS content in the chicken breast after slaughter ($P < 0.05$). Among them, the 0.3S+J group and 0.6S+J group were both lower than the 0.3S group and 0.6S group ($P < 0.05$) in the early postmortem (0–12 h), and the ROS content of the 0.3S+J group decreased respectively 2.81 and 3.62 in numerical size, the 0.6S+J group decreased by 7.32 and 5.82. It is suggested that the compound of selenium yeast and jujube powder can scavenge reactive oxygen species better than selenium yeast alone, which may be because the jujube polysaccharide in jujube powder has the effect of scavenging free radicals and can neutralize the adverse effects of large doses of selenium. Therefore, the active substances in jujube powder interacted with selenium yeast to achieve a better antioxidant effect, and there was a dose-effect ($P < 0.01$).

The Openness of Mitochondrial Permeability Transition Pore in Postmortem Chicken Breast

The effects of selenium yeast and jujube powder on the openness degree of mitochondrial permeability

Table 2. Effect of dietary selenium yeast on the selenium content of chicken breast (mg·kg⁻¹).

Items	Aging time (h)					SEM	Significance		
	0	12	24	48	72		C	T	C × T
CK	0.25 ^{De}	0.18 ^{CDf}	0.15 ^{BCd}	0.11 ^{Bd}	0.08 ^{Ae}	0.01	<0.01	<0.01	<0.01
J	0.28 ^{De}	0.22 ^{De}	0.18 ^{Cd}	0.12 ^{Bd}	0.09 ^{Ad}				
0.3S	0.49 ^{Dd}	0.39 ^{Cd}	0.32 ^{Cc}	0.30 ^{Bc}	0.24 ^{Ac}				
0.6S	0.64 ^{Db}	0.54 ^{CDb}	0.44 ^{Cb}	0.41 ^{Bb}	0.37 ^{Ab}				
0.3S+J	0.56 ^{Ec}	0.50 ^{Dc}	0.45 ^{Cb}	0.40 ^{Bb}	0.37 ^{Ab}				
0.6S+J	0.79 ^{Ea}	0.68 ^{Da}	0.60 ^{Ca}	0.51 ^{Ba}	0.44 ^{Aa}				

Note: Different capital letters indicate the significance of different maturation time under the same feeding method ($P < 0.05$); Different lowercase letters represent the difference significance between different treatment groups at the same time point ($P < 0.05$), C represents the influence of different yeast selenium supplemental level, T represents the influence of time factor, and C × T represents the influence of interaction, similarly below.

Table 3. Effect of selenium yeast and jujube powder diet on ROS content of chicken breast (fluorescence value·mg⁻¹ protein).

Items	Aging time (h)					SEM	Significance		
	0	12	24	48	72		C	T	C × T
CK	30.8 ^{Ea}	33.9 ^{Da}	35.93 ^{Ca}	38.51 ^{Ba}	39.69 ^{Aa}	0.47	<0.01	<0.01	<0.01
J	26.81 ^{Eb}	29.66 ^{Db}	33.72 ^{Cb}	35.22 ^{Bb}	37.89 ^{Ab}				
0.3S	23.57 ^{Ed}	27.82 ^{Dc}	30.81 ^{Cd}	32.82 ^{Be}	36.16 ^{Ac}				
0.6S	25.78 ^{Ec}	28.51 ^{Dc}	31.95 ^{Cc}	34.19 ^{Bb}	36.83 ^{Ac}				
0.3S+J	20.76 ^{Ee}	24.2 ^{Dd}	28.39 ^{Ce}	31.82 ^{Be}	34.76 ^{Ad}				
0.6S+J	18.46 ^{Ef}	22.69 ^{De}	25.06 ^{Cf}	28.03 ^{Bd}	30.45 ^{Ae}				

Note: Different capital letters indicate the significance of different maturation time under the same feeding method ($P < 0.05$); Different lowercase letters represent the difference significance between different treatment groups at the same time point ($P < 0.05$), C represents the influence of different yeast selenium supplemental level, T represents the influence of time factor, and C × T represents the influence of interaction, similarly below.

transition pore during postmortem aging are shown in Table 4. With the prolongation of postmortem aging, the mitochondrial permeability transition pore gradually opened. Still, adding selenium yeast and jujube powder could significantly inhibit the opening of the mitochondrial permeability transition pore ($P < 0.05$). From 0 to 72 h after slaughter, with the increase of selenium content in yeast, the inhibitory effect of preslaughter feeding on mitochondrial transition pore opening after slaughter was enhanced. In addition, the compound feeding effect of selenium yeast and jujube powder was better than that of the selenium yeast feeding group alone, and the absorbance value increased by 0.01 and 0.03, respectively, in the early postmortem period. There was a dose-effect ($P < 0.01$).

Mitochondrial Membrane Potential in Postmortem Chicken Breast

The mitochondrial membrane potential changes are shown in Table 4. The mitochondrial membrane potential of chicken breast was gradually lost with the progress of postmortem aging. Adding antioxidants such as

selenium yeast and jujube powder to the diet reduced the oxidative damage of mitochondria. It could be seen that the mitochondrial membrane potential under the 5 special feedings was significantly higher than that in the control group. Among them, the content of chicken breast in the mitochondrial membrane potential 0.6S+J group was considerably higher than that in other feeding groups ($P < 0.05$), at 72 h after slaughter, the fluorescence value measured in the 0.6S+J group was 0.95, while that in the control group was 0.99 at 0 h after slaughter. It could be seen that the addition of high doses of selenium yeast combined with jujube powder active substances in the diet made slaughtered chicken breasts show higher membrane potential and reduce mitochondrial oxidative damage during postmortem aging ($P < 0.05$), and there was a dose-effect ($P < 0.01$).

Mitochondrial Membrane Permeability in Postmortem Chicken Breast

To further understand the changes in the degree of mitochondrial oxidative damage in postmortem chicken breast meat with different diets, we investigated

Table 4. Effect of selenium yeast and jujube powder diet on mitochondrial permeability transition pore openness, mitochondrial membrane potential, mitochondrial membrane permeability of chicken breast (absorbance values/mg protein)/(ratio between the fluorescence value of JC-1 aggregate form on the fluorescence value of JC-1 monomer form)/(absorbance values).

Items	Treatments	Aging time (h)					SEM	Significance		
		0	12	24	48	72		C	T	C × T
Mitochondrial permeability transition pore	CK	0.27 ^{Ae}	0.23 ^{Be}	0.20 ^{Ce}	0.18 ^{Dd}	0.15 ^{Ed}	0.01	<0.01	<0.01	<0.01
	J	0.31 ^{Ad}	0.27 ^{Bd}	0.24 ^{Cd}	0.21 ^{Dc}	0.18 ^{Ec}				
	0.3S	0.32 ^{Acd}	0.29 ^{Bc}	0.26 ^{Cc}	0.22 ^{Dc}	0.19 ^{Eb}				
	0.6S	0.35 ^{Ab}	0.31 ^{Bb}	0.28 ^{Cb}	0.24 ^{Db}	0.20 ^{Eb}				
	0.3S+J	0.33 ^{Ac}	0.31 ^{Bb}	0.26 ^{Cc}	0.22 ^{Dbc}	0.20 ^{Eb}				
	0.6S+J	0.38 ^{Aa}	0.35 ^{Ba}	0.32 ^{Ca}	0.27 ^{Da}	0.24 ^{Ea}				
Mitochondrial membrane potential	CK	0.99 ^{Af}	0.90 ^{Bf}	0.83 ^{Cf}	0.75 ^{Df}	0.67 ^{Ef}	0.01	<0.01	<0.01	<0.01
	J	1.05 ^{Ae}	0.92 ^{Be}	0.83 ^{Ce}	0.77 ^{De}	0.71 ^{Ee}				
	0.3S	1.20 ^{Ad}	1.08 ^{Bd}	0.97 ^{Cd}	0.89 ^{Dd}	0.75 ^{Ed}				
	0.6S	1.37 ^{Ab}	1.20 ^{Bb}	1.12 ^{Cb}	1.04 ^{Db}	0.92 ^{Eb}				
	0.3S+J	1.26 ^{Ac}	1.12 ^{Bc}	1.02 ^{Cc}	0.93 ^{Dc}	0.81 ^{Ec}				
	0.6S+J	1.44 ^{Aa}	1.24 ^{Ba}	1.15 ^{Ca}	1.13 ^{Da}	0.95 ^{Ea}				
Mitochondrial membrane permeability	CK	0.12 ^{Ae}	0.10 ^{Be}	0.09 ^{Ce}	0.07 ^{De}	0.07 ^{De}	0.00	<0.01	<0.01	<0.01
	J	0.13 ^{Ad}	0.11 ^{Be}	0.10 ^{Cd}	0.08 ^{Dbc}	0.08 ^{Dd}				
	0.3S	0.15 ^{Ac}	0.13 ^{Bd}	0.12 ^{Cc}	0.10 ^{Dabc}	0.10 ^{De}				
	0.6S	0.16 ^{Abc}	0.14 ^{Ac}	0.12 ^{ABc}	0.07 ^{Cc}	0.09 ^{BCc}				
	0.3S+J	0.16 ^{Aab}	0.15 ^{Bb}	0.13 ^{Cb}	0.12 ^{Dab}	0.10 ^{Eb}				
	0.6S+J	0.17 ^{Aa}	0.16 ^{Ba}	0.15 ^{Ca}	0.12 ^{Da}	0.11 ^{Ea}				

Note: Different capital letters indicate the significance of different maturation time under the same feeding method ($P < 0.05$); Different lowercase letters represent the difference significance between different treatment groups at the same time point ($P < 0.05$), C represents the influence of different yeast selenium supplemental level, T represents the influence of time factor, and C × T represents the influence of interaction, similarly below.

mitochondrial membrane permeability. The experimentally measured absorbance values were inversely proportional to mitochondrial permeability. As shown in Table 4, the mitochondrial membrane permeability of chicken breast gradually increased with the extension of aging time. The addition of selenium yeast and jujube powder to the diet significantly inhibited the increase of mitochondrial membrane permeability ($P < 0.05$). At 24 h after slaughter, the absorbance of 0.3 and 0.6 mg·kg⁻¹ with jujube powder increased by 0.01 and 0.03, respectively, compared with adding selenium yeast alone ($P < 0.05$). It showed that feeding selenium yeast and jujube powder could significantly reduce the mitochondrial membrane permeability of chicken breast after slaughter ($P < 0.05$), and there was a dose-effect ($P < 0.01$).

Reduction Level of Cytochrome C of Chicken Breast During Aging

To further observe the oxidative damage of mitochondria under different feeding conditions during postmortem aging, the reduction level of Cyt-c was determined. There was a very significant interaction between reduction in Cyt-c reduction levels and postmortem aging time between treatments (Table 5). With the prolongation of postmortem aging time, the reduction level of Cyt-c in chicken breast meat under 6 different feeding methods decreased significantly ($P < 0.05$). At 0 h after slaughter, the compound effect of jujube powder was nonsignificant ($P > 0.05$). In contrast, at 24 to 72 h after slaughter, the decreased level of Cyt-c in chicken breast meat of selenium yeast and jujube compound group was significantly higher than that of single selenium yeast feeding group ($P > 0.05$). At 72 h after slaughter, the reduction level of Cyt-c in the compound group was 0.02

absorbance values/mg protein higher than that in the single-supplemented yeast-selenium group ($P < 0.05$).

Total Antioxidant Capacity Activity in Postmortem Chicken Breast

As seen from Table 5, with the extension of aging time, the total antioxidant capacity (T-AOC) of chicken breast meat in each feeding group gradually decreased. There was insignificant difference in T-AOC of chicken breast fed with jujube powder and 0.3 mg·kg⁻¹ selenium yeast compared with the control group during postmortem aging ($P < 0.05$), however, the T-AOC of chicken breast meat in the 3 feeding groups of 0.6S, 0.3S+J and 0.6S+J was significantly higher than that of the other groups ($P < 0.05$) at 0 to 12 h after slaughter. Among them, 12 h after slaughter, the T-AOC of 0.6S+J group reached 128.05 U/g, significantly higher than that of other feeding groups. Therefore, adding high-dose selenium yeast and jujube powder to the diet could dramatically increase the T-AOC of chicken breasts in the early postmortem period, and there was a dose-effect ($P < 0.01$).

Glutathione Peroxidase Activity in Postmortem Chicken Breast

GSH-Px is a peroxidase-degrading enzyme that is widely present in the body. Selenocysteine is its active center, and the enzyme's activity can reflect the selenium level in the body. About 30% to 40% of selenium in the body exists in the form of GSH-Px. The changes of GSH-Px in the chicken breast under different treatments are shown in Table 5. At 0 h after slaughter, the GSH-Px activity of the selenium yeast group increased to 182.24 $\mu\text{mol}\cdot\text{g}^{-1}$ and 219.13 $\mu\text{mol}\cdot\text{g}^{-1}$, respectively, compared

Table 5. Effect of selenium yeast and jujube powder diet on reduction level of Cyt-c, T-AOC activity, GSH-Px activity of chicken breast (absorbance values/mg protein)/(U/g)/($\mu\text{mol}\cdot\text{g}^{-1}$).

Items	Treatments	Aging time (h)					SEM	Significance		
		0	12	24	48	72		C	T	C × T
Reduction Level of Cyt-c	CK	0.12 ^{Ad}	0.10 ^{Bd}	0.08 ^{Cd}	0.07 ^{Dd}	0.05 ^{Ef}	0.01	<0.01	<0.01	<0.01
	J	0.13 ^{Ac}	0.11 ^{Bc}	0.10 ^{Cc}	0.07 ^{Dd}	0.06 ^{Ee}				
	0.3S	0.13 ^{Abc}	0.12 ^{Bc}	0.10 ^{Cc}	0.09 ^{Dc}	0.06 ^{Ed}				
	0.6S	0.15 ^{Aa}	0.13 ^{Bb}	0.11 ^{Cbc}	0.10 ^{Db}	0.07 ^{Ec}				
	0.3S+J	0.14 ^{Ab}	0.12 ^{Bc}	0.11 ^{Cb}	0.10 ^{Db}	0.08 ^{Eb}				
	0.6S+J	0.15 ^{Aa}	0.14 ^{Ba}	0.12 ^{Ca}	0.11 ^{Da}	0.09 ^{Ea}				
T-AOC	CK	96.43 ^{Ad}	93.01 ^{Ae}	89.60 ^{ABb}	86.37 ^{Ba}	81.69 ^{Ba}	6.28	<0.01	<0.01	<0.01
	J	103.79 ^{Ad}	97.86 ^{ABd}	90.68 ^{ABCb}	88.88 ^{Ba}	83.49 ^{Ca}				
	0.3S	111.16 ^{AcD}	102.00 ^{ABd}	93.01 ^{BCb}	89.42 ^{Ca}	85.11 ^{Ca}				
	0.6S	132.36 ^{Aab}	119.78 ^{Bb}	99.30 ^{Cb}	96.07 ^{CDa}	88.16 ^{Da}				
	0.3S+J	125.89 ^{Abc}	114.57 ^{Bc}	95.89 ^{Cb}	92.65 ^{Ca}	86.37 ^{Ca}				
	0.6S+J	146.73 ^{Aa}	128.05 ^{Ba}	111.52 ^{Ca}	97.68 ^{CDa}	90.32 ^{Da}				
GSH-Px	CK	155.21 ^{Ad}	140.89 ^{Be}	127.85 ^{Ce}	129.13 ^{Ce}	129.76 ^{Ce}	4.88	<0.01	<0.01	<0.01
	J	160.61 ^{Ad}	143.44 ^{Be}	129.44 ^{Ce}	128.49 ^{Ce}	123.72 ^{Ce}				
	0.3S	182.24 ^{Ac}	176.20 ^{Bd}	170.79 ^{Cd}	162.20 ^{Dd}	159.34 ^{Dd}				
	0.6S	219.13 ^{Ab}	204.50 ^{Bb}	192.73 ^{Cb}	183.19 ^{Db}	174.61 ^{Db}				
	0.3S+J	193.05 ^{Ac}	189.55 ^{Ac}	180.97 ^{Bc}	171.43 ^{Cc}	166.97 ^{Dc}				
	0.6S+J	242.03 ^{Aa}	217.86 ^{Ba}	210.23 ^{BCa}	201.96 ^{CDa}	194.01 ^{Da}				

Note: Different capital letters indicate the significance of different maturation time under the same feeding method ($P < 0.05$); Different lowercase letters represent the difference significance between different treatment groups at the same time point ($P < 0.05$), C represents the influence of different yeast selenium supplemental level, T represents the influence of time factor, and C × T represents the influence of interaction, similarly below.

with $155.21 \mu\text{mol}\cdot\text{g}^{-1}$ in the control group, which was 17% and 41% higher than that in the control group. This is consistent with the previous results for selenium content. With the prolongation of postmortem aging time, the activity of GSH-Px in chicken breast decreased significantly ($P < 0.05$). Compared with the control group, the GSH-Px activity in the J group increased but did not reach a significant level ($P > 0.05$), the 0.3S+J and 0.6S+J groups were significantly higher than those in the control group at 0 h after slaughter: high $37.84 \mu\text{mol}\cdot\text{g}^{-1}$ and $86.82 \mu\text{mol}\cdot\text{g}^{-1}$ ($P > 0.05$). The results showed that the single use of jujube powder had a nonsignificant effect on improving antioxidant enzyme activities. In contrast, the combined use of selenium yeast and jujube powder could significantly increase the GSH-Px activity of chicken breast after slaughter ($P < 0.05$), and there is a very significant dose-effect ($P < 0.01$).

Carbonyl Content in Postmortem Chicken Breast

The carbonyl content of chicken breast meat in each treatment group is shown in Table 6. The carbonyl content in chicken breast increased gradually with the extension of postmortem aging time. From 12 to 72 h after slaughter, adding selenium yeast and jujube powder to the diet could significantly reduce the carbonyl content in chicken breast ($P < 0.05$). Among them, at 12 h after slaughter, the carbonyl content of 0.6S+J group was $1.58 \text{ meat}/(\text{nmol}\cdot\text{mg}^{-1})$, which was significantly lower than that of other feeding groups ($P < 0.05$). The above results showed that the addition of high-dose selenium yeast and jujube powder to the diet could significantly reduce the carbonyl content of mature chicken breast after slaughter ($P < 0.05$), and there was a dose-effect ($P < 0.01$).

Sulfhydryl Content in Postmortem Chicken Breast

According to the above research, the changes of the corresponding sulfhydryl content in each feeding group were further determined. The results are shown in

Table 6. With the prolongation of postmortem aging time, sulfhydryl content in chicken breast decreased gradually. From 12 to 72 h after slaughter, adding selenium yeast and jujube powder to the diet significantly increased the sulfhydryl content in chicken breast ($P < 0.05$). At 12 h after slaughter, the sulfhydryl content of 0.3S+J and 0.6S+J groups reached $84.63 \text{ meat}/(\text{nmol}\cdot\text{mg}^{-1})$ and $100.29 \text{ meat}/(\text{nmol}\cdot\text{mg}^{-1})$. Among them, the sulfhydryl content in the high-dose selenium yeast jujube powder compound group was significantly higher than in other feeding groups ($P < 0.05$). The results showed that the addition of high-dose selenium yeast and jujube powder to the diet could significantly increase the sulfhydryl content of mature chicken breast after slaughter ($P < 0.05$), and there was a dose-effect ($P < 0.01$).

Caspase-9/3 Activity in Postmortem Chicken Breast

As shown in Figure 1, with the prolongation of postmortem aging time, the activity of Caspase-9/3 in chicken breasts in the control group first increased to 1.34 and 1.44 and then decreased gradually. The enzyme activity of the feeding group supplemented with antioxidant substances such as selenium yeast and jujube powder was lower than that of the control group. The high-dose selenium yeast and jujube powder compound group was significantly lower than the other feeding groups at 24 h after slaughter ($P < 0.05$), and the 0.6 mg selenium yeast and jujube powder compound group decreased Caspase-9/3 activity by 0.21 and 0.3 compared with the single-supplemented selenium yeast group respectively. The results showed that the compound addition of selenium yeast and jujube powder had a better effect on reducing the activities of Caspase-9 and Caspase-3 than the single addition of selenium yeast and had a specific impact on the apoptosis process of chicken.

Myofibrin Fragmentation Index in Postmortem Chicken Breast

The changes of MFI values under different treatments are shown in Table 7. The MFI values of chicken breasts

Table 6. Effect of selenium yeast and jujube powder diet on carbonyl and sulfhydryl content of chicken breast/ $(\text{nmol}\cdot\text{mg}^{-1})$.

Items	Treatments	Aging time(h)					SEM	Significance		
		0	12	24	48	72		C	T	C × T
Carbonyl	CK	1.69 ^{Ea}	2.18	2.64 ^{Ca}	3.04 ^{Ba}	3.73 ^{Aa}	0.09	<0.01	<0.01	<0.01
	J	1.51 ^{Eab}	1.85 ^{Db}	2.36 ^{Cb}	2.67 ^{Bb}	3.40 ^{Ab}				
	0.3S	1.49 ^{Eb}	1.76 ^{Db}	2.29 ^{Cb}	2.58 ^{Bbc}	3.04 ^{Ac}				
	0.6S	1.35 ^{Dbc}	1.76 ^{Cb}	2.16 ^{Cb}	2.33 ^{Bd}	2.75 ^{Ad}				
	0.3S+J	1.40 ^{Ebc}	1.85 ^{Db}	2.22 ^{Cb}	2.49 ^{Bc}	3.24 ^{Ab}				
	0.6S+J	1.24 ^{Dc}	1.58 ^{Dc}	1.95 ^{Cc}	2.27 ^{Bd}	2.45 ^{Ae}				
	Sulfhydryl	CK	77.50 ^{Ac}	51.76 ^{Be}	43.75 ^{Ce}	44.43 ^{Dc}				
J	77.72 ^{Ac}	67.57 ^{Bd}	57.13 ^{Cd}	52.35 ^{BCbc}	34.49 ^{Dd}					
0.3S	80.51 ^{Ac}	71.69 ^{Bcd}	62.79 ^{Cc}	56.84 ^{Cab}	44.85 ^{Dc}					
0.6S	80.96 ^{Ac}	74.71 ^{Bc}	64.34 ^{Cbc}	57.21 ^{Dab}	47.79 ^{Ebc}					
0.3S+J	100.96 ^{Ab}	84.63 ^{Bb}	68.16 ^{Cab}	58.31 ^{Da}	50.81 ^{Eb}					
0.6S+J	114.49 ^{Aa}	100.29 ^{Ba}	72.87 ^{Ca}	60.81 ^{Da}	53.09 ^{Ea}					

Note: Different capital letters indicate the significance of different maturation time under the same feeding method ($P < 0.05$); Different lowercase letters represent the difference significance between different treatment groups at the same time point ($P < 0.05$), C represents the influence of different yeast selenium supplemental level, T represents the influence of time factor, and C × T represents the influence of interaction, similarly below.

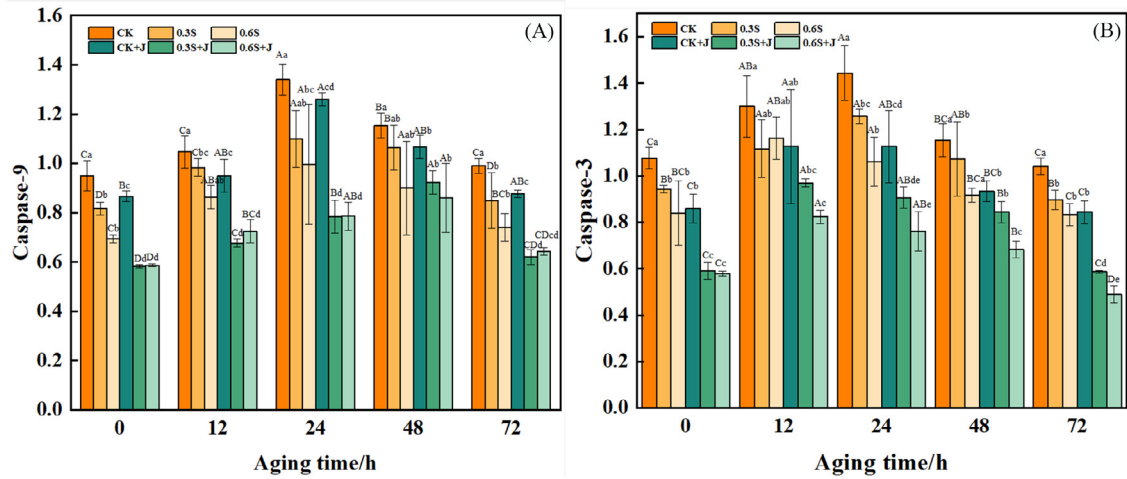


Figure 1. Effect of Selenium yeast and Jujube powder diet on caspase-9/3 activity of breast meat (enzymatic activity/ $\text{min}^{-1}\text{mg}^{-1}$).

under the 6 feeding methods gradually increased with maturity and showed an upward trend. Compared with the control group, the postmortem maturity of chicken breast in the selenium yeast group and the compound group of selenium yeast jujube powder was significantly reduced, and there was a dose-effect ($P < 0.01$). Among them, the MFI value of the high-dose compound group was considerably lower than that of the other feeding groups ($P < 0.05$), and its value ranged from 45.93 to 76.93 in the postmortem aging process, while the MFI value of the control group reached 91.2 in the late postmortem stage. The results showed that a single addition of selenium yeast in the diet could inhibit the degradation of chicken myofibrils in the early postmortem period ($P < 0.05$). The active substances in jujube powder combined with selenium yeast could significantly reduce the degradation of chicken myofibrils from 0 to 72 h after slaughter ($P < 0.05$). This difference might be due to the antioxidant effect of selenium yeast and jujube powder, which attenuated the oxidation of myofibrin, and thus significantly inhibited the degradation of myofibrin.

Shear Force in Postmortem Chicken Breast

The effects of selenium yeast and jujube powder on the shear force of chicken breast are shown in Table 7.

With the extension of aging time after slaughter, the shear force of chicken breast gradually decreased, and the meat quality was continuously tenderized. At 0 h after slaughter, the shear force value of the 0.6S+J group was 90.53 N, which was 26.4 N higher than that of the control group. At the same time, the shear force of chicken breast in 0.3S group, 0.6S group, 0.3S+J group, and 0.6S+J group was significantly higher than that in the control group ($P < 0.05$). The results showed that adding selenium yeast and jujube powder to the basal diet could significantly increase the shear force of chicken breast after slaughter, and the results were consistent with MFI.

Correlation Analysis

The correlation analysis between the degree of oxidative damage and the activity of apoptotic enzymes in the chicken breast after slaughter under different feeding methods is shown in Figure 2. Sulfhydryl content was positively correlated with MPTP, mitochondrial membrane potential, mitochondrial membrane permeability, Cyt-c reduction level, shear stress, GSH-Px, T-AOC, and se content. Carbonyl content was positively correlated with ROS and MFI. ROS was positively associated with Caspase-3, Caspase-9, and MFI ($P < 0.05$). In

Table 7. Effect of selenium yeast and jujube powder diets on myofibrin fragmentation index and shear force of chicken breast/(N).

Items	Treatments	Aging time(h)					SEM	Significance		
		0	12	24	48	72		C	T	C × T
MFI	CK	50.80 ^{Ea}	65.53 ^{Da}	72.27 ^{Ca}	84.13 ^{Ba}	91.20 ^{Aa}	0.46	<0.01	<0.01	<0.01
	J	49.60 ^{Ec}	63.33 ^{Db}	71.73 ^{Cab}	83.67 ^{Bb}	90.20 ^{Aa}				
	0.3S	50.40 ^{Eb}	61.93 ^{Dc}	71.40 ^{Cb}	82.97 ^{Bc}	90.40 ^{Aa}				
	0.6S	48.60 ^{Ed}	60.20 ^{Dd}	70.53 ^{Cc}	82.93 ^{Bc}	90.27 ^{Aa}				
	0.3S+J	47.60 ^{Ee}	56.67 ^{De}	63.27 ^{Cd}	69.53 ^{Bd}	78.07 ^{Ab}				
	0.6S+J	45.93 ^{Ef}	53.13 ^{Df}	62.53 ^{Ce}	69.40 ^{Bd}	76.93 ^{Ac}				
Shear force	CK	64.13 ^{Ad}	35.86 ^{Bd}	30.26 ^{BCd}	23.52 ^{CDc}	19.05 ^{De}	2.31	<0.01	<0.01	<0.01
	J	66.24 ^{Ad}	43.45 ^{Bc}	31.30 ^{Cd}	27.08 ^{Dd}	21.15 ^{Ede}				
	0.3S	75.20 ^{Ac}	43.60 ^{Bc}	34.02 ^{Ccd}	30.33 ^{Dc}	23.17 ^{Ed}				
	0.6S	84.36 ^{Ab}	45.32 ^{Bc}	37.39 ^{Cb}	33.11 ^{Dc}	27.41 ^{Ec}				
	0.3S+J	83.87 ^{Ab}	51.60 ^{Bb}	42.24 ^{Cb}	39.55 ^{CDb}	37.38 ^{Db}				
	0.6S+J	90.53 ^{Aa}	57.37 ^{Ba}	49.77 ^{Ca}	46.02 ^{Da}	45.61 ^{Da}				

Note: Different capital letters indicate the significance of different maturation time under the same feeding method ($P < 0.05$); Different lowercase letters represent the difference significance between different treatment groups at the same time point ($P < 0.05$), C represents the influence of different yeast selenium supplemental level, T represents the influence of time factor, and C × T represents the influence of interaction, similarly below.

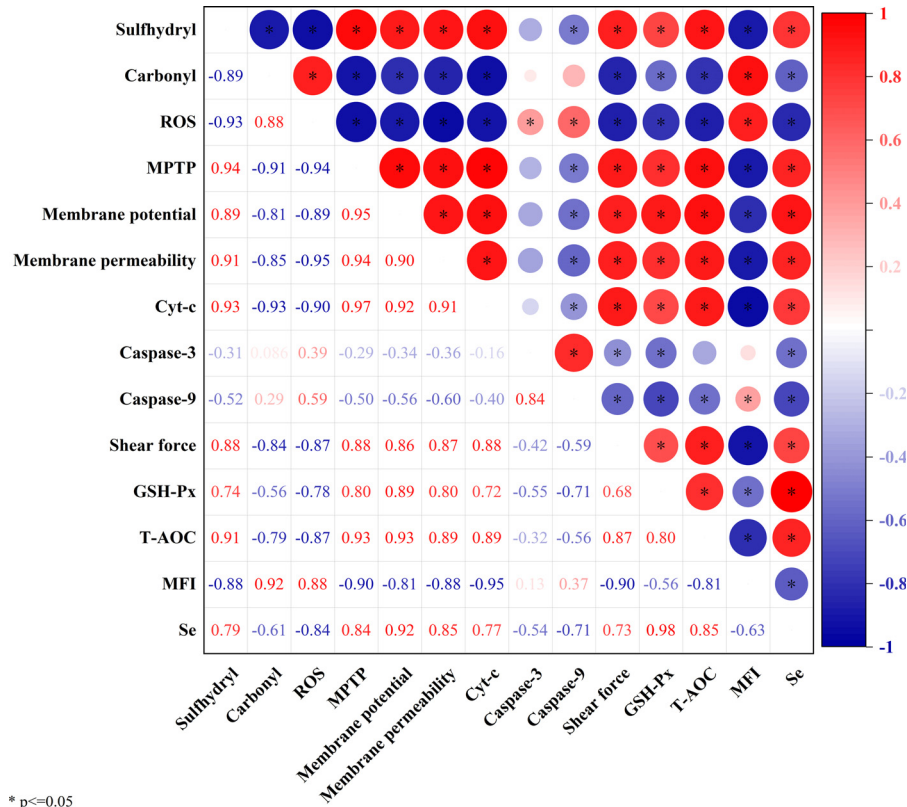


Figure 2. Correlation analysis.

addition, se content, MPTP openness, mitochondrial membrane potential, mitochondrial membrane permeability, Cyt-c reduction level, shear stress, GSH-Px, and T-AOC were significantly positively correlated ($P < 0.05$). These results indicated that adding selenium yeast and jujube powder to the basal diet could affect chicken breast cell apoptosis by modulating the body's antioxidant system after slaughter and had a significant dose effect.

Effects of Selenium Yeast and Jujube Powder on Mitochondrial Oxidative Damage and Apoptosis of Postmortem Broilers Based on Principal Component Analysis

The results of principal component analysis based on mitochondrial oxidative damage and apoptotic enzyme activity are shown in Figure 3; PC1 and PC2 explained 78.07% and 12.81% of variance contribution rate, respectively, and accumulated to 90.88%, which reflected most of the initial information of 14 related indicators in the process of chicken ripening under 6 feeding methods. Therefore, the first 2 principal component factors can be extracted for discussion and analysis, and the analysis results are shown in the figure. The component loads of each index can be obtained from Table 8. The one with a significant absolute value indicated a high correlation between the principal component and index, and the plus and minus signs showed positive and negative correlations. PC1 mainly synthesized ROS, carbonyl group, sulphydryl group, MPTP,

mitochondrial membrane potential, mitochondrial membrane permeability, Cyt-c reduction level, T-AOC, and se content, and mainly represented the oxidative damage degree of postmortem chicken breast, so PC1 was defined as an oxidation factor. PC2 primarily synthesizes Caspase-3 and Caspase-9 and mainly represents the activity of apoptotic enzymes in chicken breast, so PC2 was defined as an apoptotic factor. PC1 was

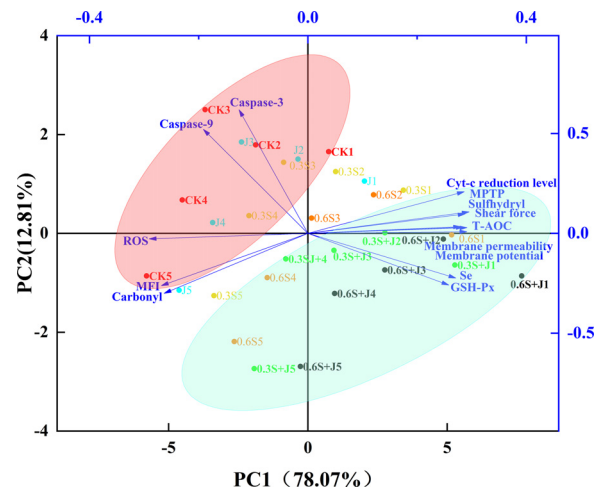


Figure 3. Principal component analysis biplot. Note: The last digit 1-4 represents 0 h after slaughter and 12, 24, 48, and 72 h after maturity, respectively. CK before this digit represents control group, 0.3S represents 0.3 mg·kg⁻¹ selenium yeast group, 0.6S represents 0.6 mg·kg⁻¹ selenium yeast group, J represents jujube powder group, 0.3S+J group was 0.3 mg·kg⁻¹ Selenium yeast and jujube powder compound group, 0.6S+J group was 0.6 mg·kg⁻¹ Selenium yeast and jujube powder compound group.

Table 8. Feature vector, eigenvalue, contribution rate, and cumulative contribution rate of principal components.

Items	Sulfhydryl	Carbonyl	ROS	MPTP	Membrane potential	Membrane permeability	Cyt-c	Caspase-3	Caspase-9	Shear force	GSH-Px	T-AOC	MFI	Se	Eigenvalue	Contribution%	Cumulative contribution rate %
PC1	0.29	-0.26	-0.29	0.30	0.29	0.29	0.29	-0.13	-0.20	0.28	0.26	0.28	-0.27	0.27	10.93	78.05	78.05
PC2	0.10	-0.30	-0.03	0.11	0.01	0.03	0.21	0.62	0.52	0.03	-0.26	0.03	-0.26	-0.21	1.79	12.81	90.86

positively correlated with sulfhydryl, mitochondrial pore opening degree, mitochondrial membrane potential, mitochondrial membrane permeability, Cyt-c reduction level, shear force, GSH-Px activity, total antioxidant capacity, and selenium content; meantime, the correlation between PC2 and Caspase-3 and Caspase-9 was high, these results were consistent with the previous correlation analysis results in Figure 2.

The biplot of principal component analysis for selenium content, oxidation, and antioxidant indexes, and tenderness changes of chicken during aging under different feeding methods is shown in the figure. In the process of chicken aging, carbonyl, sulfhydryl, selenium content, GSH-Px activity, total antioxidant capacity, MPTP openness, mitochondrial membrane potential, mitochondrial membrane permeability, shear force, and Cyt-c reduction level changed significantly. The activity of Caspase-3 and Caspase-9 changed significantly at 0 to 24 h of aging. However, ROS levels, carbonyl content, and MFI values change significantly at 48 to 72 h of maturity, consistent with the results in Table 2 to 7 and Figure 1. It can be seen from the figure that with the extension of aging time, each feeding group gradually moved from the positive quadrant of PC1 to the negative quadrant of PC1. From the corresponding indicators, the content of selenium in chicken decreased, the degree of mitochondrial oxidation increased, apoptosis occurred, and meat tenderness became better. The 6 feeding groups were evenly distributed from left to right. The results showed that with the increase of dietary selenium yeast content, the content of selenium in chicken breast increased, the antioxidant performance of chicken was improved, and the activity of apoptotic enzymes was decreased, thus inhibiting the tenderness of the chicken. At the same time, the effect of high-dose selenium yeast on reducing mitochondrial oxidative damage was more significant, and this showed that under the action of jujube powder, the concentration of selenium yeast in the diet has a specific positive correlation with the antioxidant capacity of broilers after slaughter.

DISCUSSION

The trace element selenium can be added in the form of inorganic selenium or organic selenium. In addition, it can also be compounded with plant-derived substances such as red dates that contain natural antioxidant substances and antioxidant elements. Supplementation of the trace element selenium in the diet can significantly increase the deposition of selenium in the muscles of animals after slaughter because the selenomethionine in organic selenium can penetrate protein molecules and be well deposited in animals (Wan et al., 2019). The evidence suggested that adding $0.3 \text{ mg}\cdot\text{kg}^{-1}$ selenium yeast to the diet increased the selenium content of the chicken breast of Hy-Line Brown hens by 299.44% compared with the control group (Lu et al., 2019). The above research results showed that selenium yeast feeding significantly increased the selenium content in chickens

after slaughter. In addition, recent observations have proposed that adding sodium selenite and selenium-enriched *Saccharomyces cerevisiae* to the basal diet could significantly increase the deposition of selenium in broilers after slaughter. The effect of the selenium-enriched *Saccharomyces cerevisiae* feeding group was considerably higher than that of other feeding groups (Hou et al., 2020). This research found that adding different concentrations of selenium yeast and jujube powder to the diet, as well as the combination of the two, could improve the enrichment of selenium in chicken after slaughter to various degrees. When $0.3 \text{ mg}\cdot\text{kg}^{-1}$ or 0.6-kg^{-1} selenium yeast was added alone, the content of selenium in broilers at 0 h after slaughter was increased by 0.24-kg^{-1} and 0.39-kg^{-1} , respectively, compared with the control group. Interestingly, when selenium yeast was compounded with jujube powder, the selenium content in the chicken breast after slaughter was significantly higher than that in the single selenium yeast feeding group, which increased by 23.44% and 14.29%, respectively. These results indicated that the compounding of selenium yeast and jujube powder significantly increased the selenium content in chicken after slaughter. This is because red dates are rich in trace elements, minerals, and their unique active substances, jujube polysaccharides (Ji et al., 2020). While these exert their antioxidant effects, the compound addition of selenium yeast can better enrich the selenium content in chicken breast compared to the single addition of selenium yeast.

The self-oxidative and anti-oxidative imbalance of postmortem animals, and excessive accumulation of ROS will lead to a series of oxidative stress damage in cells. Ren et al. found that selenium pretreatment could significantly inhibit ROS content in mouse cells. In this experiment (Ren et al., 2020), the ROS level and carbonyl content in chicken breast meat in the compound feeding group of selenium yeast and jujube powder were significantly lower than those in the other 4 feeding groups, while the sulfhydryl content was higher than the rest of the feeding groups. This indicated that adding selenium yeast and jujube powder to the diet could significantly reduce the ROS level in the chicken breast by inhibiting cellular peroxidation, thereby attenuating the oxidative stress of mitochondria. In addition, this study found that the GSH-Px activity of chicken breast in the yeast-selenium-supplemented diet groups were significantly higher than that in the control group during postmortem aging, and there was a dose-effect. These results are supported by the previous study that showed selenium yeast significantly improved the antioxidant capacity of dairy cows, as shown in the significant increase in serum GSH-Px activity and total antioxidant capacity, which is similar to the results of this study (Sun et al., 2021). This is because selenium is the active component of GSH-Px, and selenium yeast feeding enhances the activity of GSH-Px by enhancing the enrichment of selenium in chicken after slaughter (Rotruck et al., 1973). GSH-Px, as an endogenous antioxidant enzyme, can be involved in the animals. The

peroxidation defense system, which is widely present in animal bodies, can catalyze the formation of oxidized glutathione (GSSG) from glutathione (GSH), thereby protecting the structure and function of cell membranes (Mahmoud and Edens, 2003). In addition, the total antioxidant capacity of chicken breast meat in 0.6S group and 0.6S+J group was significantly higher than that in other feeding groups in the early postmortem stage. Still, it was nonsignificant with the extension of aging time. This is because the excessive ROS produced by cells will destroy the integrity of the cell membrane with the progress of the body's metabolism, and animals have both enzyme-induced and nonenzyme-induced antioxidants. However, selenium yeast and jujube powder feeding could increase the content of selenoprotein in chicken after slaughter, thereby increasing the peroxidase activity in vivo. In addition, jujube polysaccharides in red dates have the function of scavenging excess free radicals in cells, indirectly improving the tolerance of animals to hypoxia in the early postmortem period and reducing oxidative damage (Mousaie, 2021). Therefore, under the dual effect of high-dose selenium yeast and jujube powder, the antioxidant capacity of chicken breast in the early postmortem can be improved (Zhang et al., 2021).

The occurrence of apoptosis is affected by many factors, among which ROS has been confirmed to be one of the essential inducements of apoptosis (Yan et al., 2022). Some studies indicated that under stress, cells produced a large amount of ROS, which promoted the conformation of MPTP between the inner and outer mitochondrial membranes to change, and various pro-apoptotic factors were released from the pores in large quantities, eventually triggering the apoptosis cascade (Li et al., 2018; Feng et al., 2022). Wang et al. reported that mitochondrial membrane potential decreased significantly during postmortem aging of yak meat, which was consistent with the results of this experiment (Wang et al., 2018). In this study, adding selenium yeast to the diet improved mitochondrial membrane potential and membrane permeability. There was a dose-effect, suggesting that selenium yeast feeding could protect mitochondrial structure and function by reducing mitochondrial oxidative damage. Recent studies also found that treatment with selenomethionine in vitro could significantly increase selenoprotein and membrane potential in bovine mammary glands and reduce the expression of Cyt-c, Caspase-3, and Caspase-9 proteins (Zhuang et al., 2021). In addition, this study found that adding selenium yeast to the diet could significantly inhibit the opening degree of MPTP and enhance substantially the reduction level of Cyt-c in the cytoplasm. Jin et al. found that supplementing $0.2 \text{ mg}\cdot\text{kg}^{-1}$ of selenium (in the form of Na_2SeO_3) in the basal diet could significantly reduce the activity of Caspase-3 and the expression of pro-apoptotic protein Cyt-c in the pancreas of Hylan brown laying hens after slaughter (Jin et al., 2018). This is because selenium supplementation can significantly inhibit the release of pro-apoptotic protein Cyt-c and delay the process of apoptosis (Lee and Lee, 2018). At the same time, the research found that

adding sodium selenite to the diet might reduce the activity of chicken Caspase-3 by improving the oxidative stress of Hy-Line brown hens (Chen et al., 2017a). The results were consistent with the activity of Caspase-3 and Caspase-9, indicating that the selenium-supplemented diet inhibited the activation of Caspase-9 by reducing Cyt-c, thereby reducing the activity of the downstream apoptosis effector enzyme Caspase-3. Eventually, the apoptosis process was delayed.

Mitochondrial mediated endogenous apoptosis pathway is one of the 3 activation pathways of caspase-mediated apoptosis, mainly involving the activation of Caspase-3 and Caspase-9. Endogenous apoptotic enzymes have been shown to be involved in postmortem muscle tenderization (Ouali et al., 2006). The postmortem aging process is the process of muscle-to-meat transformation, and MFI can be used as an indicator to reflect the integrity of muscle fibers and myofibrillar skeletal proteins (Li et al., 2014). Meanwhile, from a macro perspective, shear force can reflect the tenderness of meat (Taylor et al., 1995). In this study, the addition of selenium yeast and jujube powder to the diet can significantly reduce the MFI and increase the shear force of chicken breast after slaughter, which is consistent with the research of Vieira et al. (2021), this may be because the supplementation of natural antioxidants such as selenium yeast and jujube powder in the diet inhibited the activity of apoptotic enzymes and thus inhibited the degradation of chicken myofibrils by other means. However, studies have also shown that basal diets supplemented with different concentrations of selenium (in the form of hydroxy-selenomethionine) reduced postmortem shear stress in yellow feathered broilers (Tang et al., 2021). Unlike the changes in chicken tenderness in this study, this may be because the type of chicken and the basal diet lead to differences in the experimental results. From what has been discussed above, the mechanism of the effect of selenium yeast feeding on the tenderness of broiler chicken breast after slaughter remains to be further studied. Therefore, although the feeding of selenium yeast and jujube powder can improve the antioxidant capacity of chickens after slaughter, at the same time, it also delays the aging of chickens by inhibiting the apoptosis of the mitochondrial pathway, which has a particular effect on the tenderization of chickens. However, the mechanism of its impact on chicken tenderness is still unclear and needs further study.

CONCLUSION

The complete results showed that dietary supplementation of selenium yeast and jujube powder significantly increased GSH-Px activity and weakened mitochondrial oxidative damage, thus protecting mitochondrial structure and function, and the combined effect of selenium yeast and jujube powder at 0.6 mg·kg⁻¹ was more significant. Moreover, it was found in this study that the addition of selenium yeast and jujube powder delayed cell apoptosis, inhibited the degradation of myofibrin

protein to a certain extent, and thus delayed the aging process of chicken, keeping the tenderness of chicken at an acceptable range.

ACKNOWLEDGMENTS

Funding: This research was funded by the National Natural Science Foundation of China (No. 32060550).

DISCLOSURES

The authors declare they have no conflict of interest.

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