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# Effect of *Rosa damascena* on Serum Oxidant/ Antioxidant Status in Chickens with Pulmonary Hypertension

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## Abstract

**Background and Objective:** This study evaluated the antioxidant effects of Rosa damascena (rose) powder on the serum oxidant/antioxidant balance in broiler chickens with cold stress-induced pulmonary hypertension.

**Materials and Methods:** A total of 192 one-day-old Ross 308 chickens were allocated to four groups: (1) a negative control group fed a basal diet under standard temperature conditions; (2) a positive control group fed a basal diet under cold stress; and (3) rose-0.6% and (4) rose-1.2% groups, which received the basal diet supplemented with 0.6% and 1.2% rose powder, respectively, under cold stress. At 35 days of age, blood samples were collected to measure serum malondialdehyde (MDA) levels and the activities of catalase (CAT) and superoxide dismutase (SOD).

**Results:** Findings showed that MDA level was increased in the positive control group compared to the negative control group, while SOD activity was decreased ($P<0.05$). A significant reduction in serum MDA levels was observed in both rose-supplemented groups relative to the positive control group ($P<0.05$). Although CAT activity did not differ significantly ($P>0.05$), SOD activity increased in the 0.6% and 1.2% rose groups compared to the positive control ($P<0.05$). Furthermore, the right ventricular-to-total ventricular weight (RV/TV) ratio, an indicator of pulmonary hypertension syndrome, was significantly lower in the rose-0.6% supplemented groups ($P<0.05$).

**Conclusion:** These findings suggest that dietary supplementation with *Rosa damascena* powder dose-dependently improves the serum oxidant/antioxidant balance and mitigates pulmonary hypertension syndrome in broiler chickens.

**Keywords:** Antioxidant Enzymes, Broiler Chickens, Malondialdehyde, *Rosa damascena*

## 1. Introduction

Exposure to cold temperatures triggers a complex physiological response in broiler chickens as they attempt to maintain their core body temperature within the narrow range required for optimal metabolic function (1). When environmental temperatures drop below the thermoneutral zone, the birds immediately activate multiple compensatory mechanisms (2). Cutaneous thermoreceptors detect temperature change and signal the hypothalamic thermoregulatory center, which in turn stimulates

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the sympathetic nervous system. This neural activation leads to a cascade of metabolic and cardiovascular adjustments, beginning with increased secretion of thyroid hormones and catecholamines (3).

The cardiovascular system undergoes significant adaptations to support the increased metabolic demands while minimizing heat loss. Peripheral vasoconstriction reduces blood flow to the skin, effectively decreasing heat dissipation from the body surface (4). Concurrently, heart rate increases cardiac output rises to maintain adequate circulation despite the peripheral resistance. These hemodynamic changes result in a systemic blood pressure increase. The respiratory system responds to the heightened metabolic rate with increased oxygen consumption (1). This elevated demand for oxygen, combined with the pulmonary vasoconstriction that occurs in response to both the cold stress and the associated hypoxia, leads to an increase in pulmonary arterial pressure characteristic of pulmonary hypertension syndrome (PHS) (5, 6). A combination of increased cardiac output against the constricted pulmonary vasculature creates an overload and substantial stress on the right ventricle, leading to hypertrophy and dilation (7). Simultaneously, the cold stress induces significant oxidative stress at the cellular level (8). Mitochondrial reactive oxygen species (ROS) production increases as the electron transport chain works harder to meet the elevated ATP demands. NADPH oxidase activity in pulmonary vessels becomes more pronounced, further contributing to ROS generation. The resulting oxidative damage is particularly evident in the accumulation of lipid peroxidation products such as MDA (9). This condition could lead to the pathological remodeling of the heart and blood vessels (10).

*Rosa damascena* (rose) is a medicinal plant with biologically active compounds that exert significant physiological effects across various body systems. The plant contains terpenes (citronellol, geraniol), flavonoids (quercetin, kaempferol), phenolic acids (gallic acid, ellagic acid), aromatic compounds (phenylethyl alcohol), and vitamins (C, E) (11). Its antioxidant effects include free radical scavenging, reducing malondialdehyde levels, and enhancing antioxidant enzyme activity (12). The plant protects against lipid peroxidation in cell membranes and demonstrates anti-inflammatory effects (13). Cardiovascular benefits include lowering systolic blood pressure, improving endothelial function, and regulating heart rate. Neuroactive effects involve increasing serotonin and dopamine levels, reducing cortisol, and exhibiting anxiolytic properties through GABA receptors (14). Metabolically, it lowers blood glucose, improves lipid profile by reducing triglycerides and increasing HDL, while modulating liver enzymes (15). Respiratory effects include bronchodilatory action, reduced airway resistance, and improved tissue oxygenation (16). This study aimed to evaluate the effects of dietary *Rosa damascena* supplementation on serum oxidant/antioxidant balance and PHS development in cold-stressed broilers, leveraging its documented capacity to enhance antioxidant defenses and mitigate oxidative injury.

## 2. Materials and Methods

### 2.1. Bird management and induction of cold stress

The study protocol was approved by the Institutional Animal Care and Use Committee of Shahrekord University (IR.SKU.REC.1400.07001). A total of 192 one-day-old Ross 308 broiler chicks were purchased from Sepidmakian Co. and randomly assigned to four groups of 48 birds each. The first group was fed the basal diet under standard temperature conditions (negative control). The second group was maintained on a basal diet under cold stress throughout the study (positive control). Two additional groups received the basal diet supplemented with rose powder at levels of 0.6% and 1.2%, respectively, while being exposed to cold stress during the rearing period. Environmental factors including temperature, humidity, ventilation, and lighting were kept consistent for all groups until day 8. From day 8 onward, all groups except the negative control were subjected to cold stress. The ambient temperature started at 30°C on day 1 and was gradually lowered from day 8 to induce PHS, reaching 15°C by day 15 and maintained at this level until the end of the experiment (17).

### 2.2. Sampling and calculation of RV/TV ratio

On 35 days of age, 10 birds from each group were randomly selected for blood sampling. Blood samples (3 mL) were collected from the wing vein of each bird. The samples were centrifuged at 2500 rpm for 10 minutes to separate the serum. The obtained sera were transferred into 2 ml microtubes and stored at -20°C until biochemical analyses were performed.

Following blood collection, all chickens (48 birds per group) were euthanized by decapitation at 35 days of age, and their hearts were excised. The ventricles were separated and weighed, and the RV/TV ratio was calculated that serves as an index of PHS severity and right ventricular hypertrophy/ dilation. A higher RV/TV ratio indicated a more advanced progression of PHS (18).

### 2.3. MDA measurement

MDA, a primary biomarker of lipid peroxidation, was quantified in plasma samples using the thiobarbituric acid reactive substances (TBARS) assay, following the method previously described (19, 20). In brief, 100 µL of plasma was mixed with 200 µL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of sodium acetate buffer (3.5 M, pH 4.0), and 1.5 mL of 0.8% thiobarbituric acid. The mixture was heated at 95°C for 1 hour, then cooled on ice for 30 minutes. After centrifugation at 1,500 × g for 10 minutes at 4°C, the absorbance of the supernatant was measured at 532 nm. A standard curve was prepared simultaneously to quantify MDA levels, and the results were expressed in µmol/L.

### 2.4. Measurement of CAT and SOD activities

Serum activities of SOD and CAT were determined using commercially available assay kits (Nasdox and Nactaz, Navand Salamat Co., Iran) according to the manufacturer's protocols. SOD activity was measured by the nitroblue tetrazolium (NBT) reduction method, a widely used indirect assay for quantifying SOD in biological samples. In this assay, superoxide radicals generated in the reaction system reduce NBT to a blue formazan dye. The presence of SOD in the serum inhibits this reduction by catalyzing the dismutation of superoxide anions into hydrogen peroxide and molecular oxygen, thereby decreasing the intensity of the blue color. The degree of inhibition of formazan formation is directly proportional to SOD activity and was quantified by measuring absorbance at 560 nm using a spectrophotometer. Results were expressed as units per milliliter (U/mL) of serum.

CAT activity was assessed using the ammonium molybdate method, following the kit instructions. This method is based on the ability of catalase to decompose hydrogen peroxide ($H_2O_2$) present in the reaction mixture. After the reaction, ammonium molybdate is added to react with the remaining $H_2O_2$, forming a yellow complex. The decrease in yellow color intensity, measured at 405 nm, reflects the catalase activity in the sample. Results were expressed as units per milliliter (U/ml) of serum.

### 2.5. Statistical analysis

Data are presented as means ± standard error of the mean (SEM). Normality of distribution was confirmed using the Shapiro-Wilk test. Statistical comparisons between experimental groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for pairwise comparisons, implemented in SPSS software (Version 26.0, IBM Corp., USA).

## 3. Results

### 3.1. Analysis of RV/TV ratios

The RV/TV ratio was increased in the positive control group compared to the negative control and rose-0.6% supplemented groups ($P < 0.05$, Figure 1A). No significant differences were observed among the negative control, rose-0.6%, and rose-1.2% supplemented groups ($P > 0.05$, Figure 1A).

### 3.2. Analysis of MDA levels

The MDA level was increased in the positive control group compared to the negative control, rose-0.6% and rose-1.2% supplemented groups ($P < 0.05$, Figure 1B). The MDA level in the rose-0.6% supplemented group was significantly lower than other experimental groups ($P < 0.05$, Figure 1B).

### 3.3. Antioxidant enzyme (SOD and CAT) responses

The CAT activity did not statistically differ among different experimental groups of chickens ($P > 0.05$, Figure 2A). The SOD activity was lower in the positive control group than the negative control and rose-0.6% and rose-1.2% supplemented groups ($P < 0.05$, Figure 2). No statistically significant differences were observed among the negative control group, rose-0.6%, and rose-1.2% supplemented groups ($P > 0.05$, Figure 2B).

## 4. Discussion

The findings demonstrate that dietary supplementation with *Rosa damascena* powder can effectively improve the serum oxidant/antioxidant balance and reduce PHS in cold-stressed broiler chickens. Previous studies have

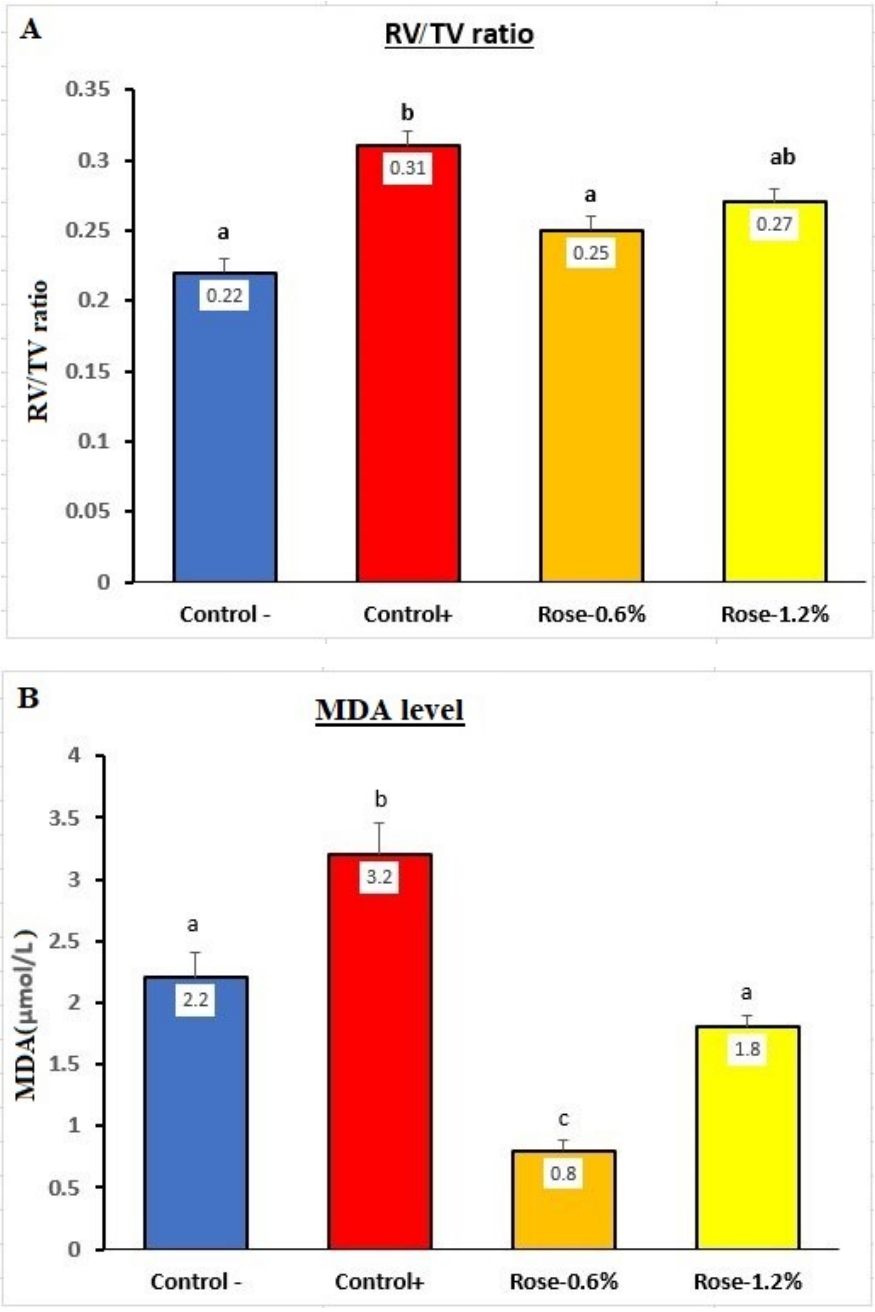


**Fig. 1.** Effects of Rosa damascena supplementation on (A) right ventricular-to-total ventricular weight ratio (RV/TV) and (B) serum malondialdehyde levels (MDA, µmol/L) in cold-stressed broilers. Groups: Negative control (Control-, standard diet, normal temperature), Positive control (Control+, standard diet, cold stress), Rose-0.6% (Control+ supplemented by 0.6% rose powder), and Rose-1.2% (Control+ supplemented by 1.2% rose powder). Data represent mean ± SEM (n=10/group). Different lowercase letters indicate significant differences ($P < 0.05$) by one-way ANOVA with Tukey's post hoc test.

consistently shown that rose possesses significant antioxidant properties, primarily attributed to its rich content of phenolic compounds, flavonoids, and other bioactive molecules (11, 21). Our observation of reduced malondialdehyde levels in rose-supplemented groups aligns well with these earlier findings, confirming the plant's ability to mitigate lipid peroxidation under oxidative stress conditions. However, the current study reveals an interesting dose-response relationship

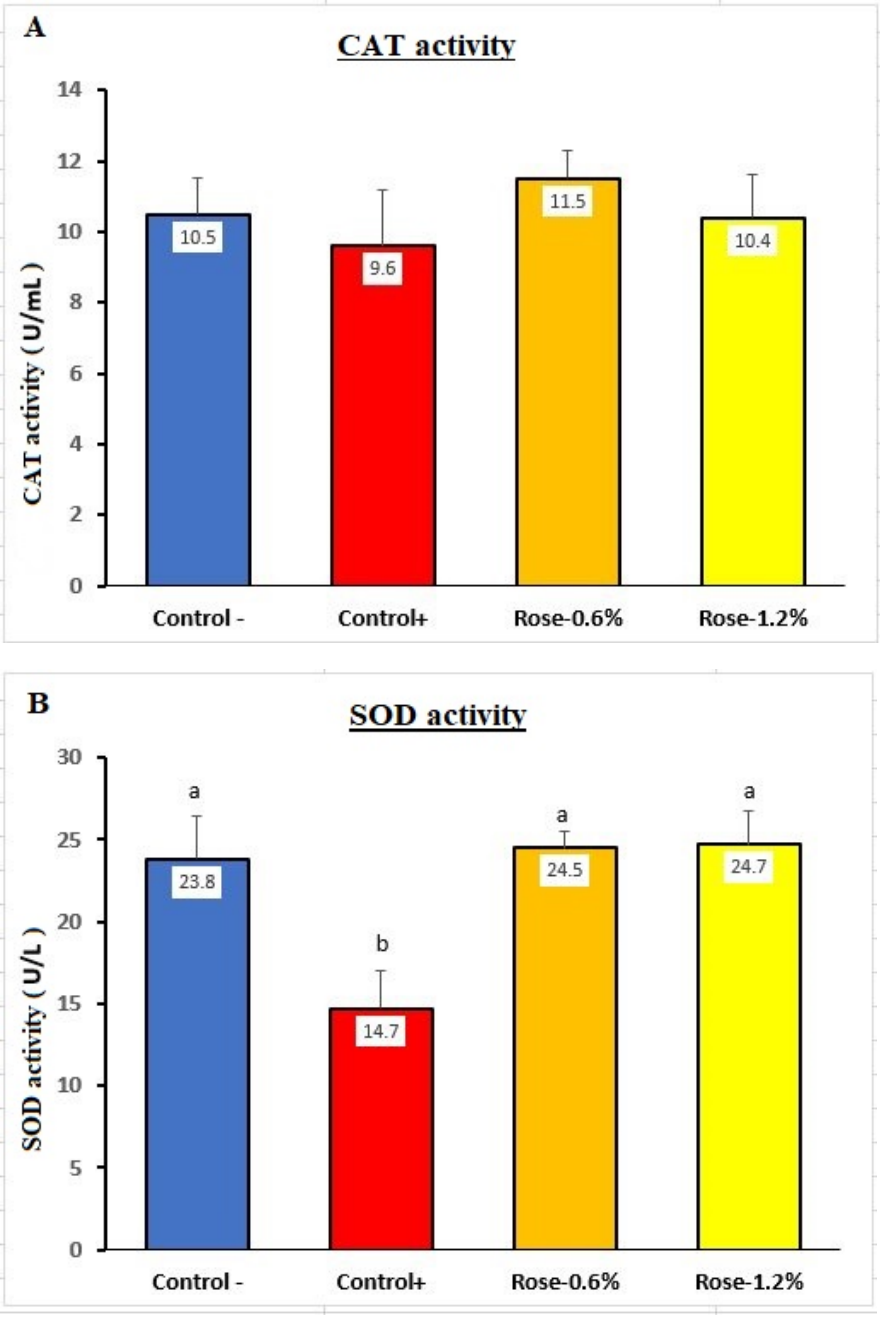


**Fig. 2.** Serum antioxidant enzyme activities in experimental groups: (A) catalase (CAT, U/mL) and (B) superoxide dismutase (SOD, U/L). Groups: Negative control (Control-, standard diet, normal temperature), Positive control (Control+, standard diet, cold stress), Rose-0.6% (Control+ supplemented by 0.6% rose powder), and Rose-1.2% (Control+ supplemented by 1.2% rose powder). Data represent mean ± SEM (n=10/group). Different lowercase letters indicate significant differences ($P < 0.05$) by one-way ANOVA with Tukey's post hoc test.

not previously reported, where the 0.6% supplementation level appeared more effective in reducing MDA than the higher 1.2% dose. This non-linear response suggests a biphasic pattern in the antioxidant effects of rose, possibly due to saturation of absorption mechanisms or pro-oxidant effects at higher concentrations (22).

The enzyme activity patterns observed in this study present both confirmatory and novel findings compared to existing literature. While

many previous reports have documented increases in both SOD and CAT activities following rose supplementation (23), our results showed a selective enhancement of SOD activity without significant changes in CAT. The differential response of SOD and CAT activities in this study reflects their specialized roles in the antioxidant defense system. While SOD serves as the primary enzyme neutralizing superoxide radicals ($O_2^{\bullet-}$), its increased activity in rose-supplemented groups suggests enhanced first-line protection against cold-stress-induced oxidative damage. This preferential stimulation of SOD may be particularly beneficial in cold stress conditions, as this enzyme serves as the first line of defense against superoxide radicals that are abundantly generated during increased metabolic activity. The effect could be attributed to several factors, including the specific composition of the rose powder used, the nature of oxidative stress induced by cold exposure, or potential differences in the temporal dynamics of enzyme induction. In contrast, the unchanged CAT activity implies that hydrogen peroxide ($H_2O_2$) decomposition, either through alternative pathways (e.g., glutathione peroxidase) or lower $H_2O_2$ production, was sufficient to maintain redox balance. This selective SOD upregulation, likely driven by *Rosa damascena*'s flavonoid constituents (e.g., quercetin), underscores its efficacy in targeting early oxidative cascades, thereby reducing lipid peroxidation without necessitating CAT involvement. (24).

Regarding the cardioprotective effects, our findings of reduced right ventricular hypertrophy/dilation (as indicated by the RV/TV ratio) in rose-supplemented groups extend previous observations of rose's cardiovascular benefits. While earlier studies have primarily focused on the plant's hypotensive and vasodilatory properties in mammalian models (25, 26), the current results demonstrate its potential to prevent pathological cardiac remodeling in poultry under cold stress. This effect likely stems from the combined action of reduced oxidative stress and improved antioxidant capacity, which collectively help maintain pulmonary vascular homeostasis (23). The comparison with prior research also highlights important methodological considerations. Many previous studies investigating rose's effects have used extracts or isolated compounds, whereas our study employed whole rose powder. This difference in preparation may account for some of the observed variations in biological activity, as the complete phytochemical matrix present in the whole powder could produce different effects compared to purified components (27). Additionally, the cold stress model used in this study represents a distinct physiological challenge that may elicit different responses compared to other oxidative stress models employed in earlier research.

These findings have important practical implications for poultry production. The demonstration that relatively low levels of rose powder supplementation (0.6%) can effectively mitigate cold-stress-induced PHS suggests a potentially cost-effective nutritional strategy for improving broiler health in challenging environmental conditions. The observed effects on the oxidant/antioxidant balance also support the potential use of rose as a natural alternative to synthetic antioxidants in poultry diets.

While this study provides compelling evidence for the antioxidant benefits of *Rosa damascena* in cold-stressed broilers, many limitations should be acknowledged. First, the experiment was conducted under controlled environmental conditions, which may not fully replicate commercial poultry farm variability. Second, we focused on serum oxidative markers (MDA, SOD, CAT) but did not assess tissue-specific antioxidant responses or gene expression patterns that could provide mechanistic insights. Third, the study duration was limited to 35 days; longer-term effects of rose powder supplementation on bird health and productivity remain to be investigated.

## Conclusion

This study demonstrates that dietary *Rosa damascena* powder effectively reduces oxidative stress and PHS in cold-stressed broilers by lowering MDA levels, enhancing SOD activity, and significantly decreasing the RV/TV ratio as key indicator of PHS severity. The 0.6% dose showed optimal efficacy in both improving antioxidant status and preventing right ventricular hypertrophy/dilation, suggesting its potential as a natural therapeutic supplement in poultry diets to mitigate cold stress-induced PHS and related cardiovascular disorders.

## Acknowledgment

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## Compliance with ethical standards

All the procedures in this study were approved by the Institutional Animal Care and Use Committee of Shahrekord University.

## Conflicts of interest

The authors declare that they have no competing interests.

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