Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

e-ISSN: 1806-9061 2026 / v.28 / n.1 / 001-009

http://dx.doi.org/10.1590/1806-9061-2025-2184

Original Article

A Study on the Preventive Mechanism of Salidroside on PAH Syndrome in Broilers

■Author(s)

Yourong Y[I,III,†] https://orcid.org/0009-0005-5719-4349
Mudan S[I,II,†] https://orcid.org/0009-0000-9641-8070
Ziqin Z[I,II,†] https://orcid.org/0009-0000-9158-0239
Sheng K[I,II] https://orcid.org/0009-0001-4495-4231
Qinxia W[I,III] https://orcid.org/0000-0002-8818-6107
Zhenyu C[I,II] https://orcid.org/0000-0001-7306-3641

[I] Xizang Agricultural and Animal Husbandry University, College of Animal Science, Linzhi, 860000, Xizang, China.
[II] Xizang Agricultural and Animal Husbandry University, Key Laboratory of Clinical Veterinary Medicine in Xizang, Nyingchi, Xizang, 860000, People's Republic of China.
[III] The Provincial and Ministerial Co-founded Collaborative Innovation Center for R&D in Xizang Characteristic Agricultural and Animal Husbandry Resources, Linzhi, 860000, Xizang, China.
[†] These authors have contributed equally.

■Mail Address

Corresponding author e-mail address
Zhenyu Chang
Xizang Agricultural and Animal Husbandry University, College of Animal Science, Linzhi, 860000, Xizang, China.
Phone: +86 176 8954 8312
Email: 1782492444@qq.com

■Keywords

CaSR, PAH, pulmonary vascular remodeling, salidroside.

Section Editor: Ramon Malheiros

Submitted: 29/October/2025
Approved: 26/December/2025

ABSTRACT

This study explored how salidroside alleviates pulmonary arterial hypertension (PAH) and ascites in broilers exposed to high-altitude hypoxia. Three groups of broilers (n = 80 each) were kept for 42 d under hypoxic (14.0–16.0 % O_2), normoxic (20.8–21.5 % O_2) or hypoxic + salidroside (50 mg kg^{-1} diet) conditions. On days 21, 35 and 42, mortality was recorded; serum SOD, GSH-Px and CAT activities, and MDA levels were quantified, and the right ventricular hypertrophy (RV/TV) index was calculated. At day 42, pulmonary arteries were sampled for histopathology, immunohistochemistry (Caspase-3, Ki67, PCNA), Western blot, RT-qPCR, and immunohistochemistry for CaSR. Compared with the hypoxic group, salidroside and normoxic broilers showed higher antioxidant enzyme activities, lower MDA, lower RV/TV index, and reduced total and ascites-related mortality (all $p<0.05$). Hypoxia-induced thickening of the arterial wall, decrease in Caspase-3, and increases in Ki67 and PCNA were all reversed by salidroside or normoxia. CaSR mRNA and protein levels were also downregulated in both non-hypoxic groups ($p<0.05$), with no difference between them ($p>0.05$). Salidroside thus mitigates hypoxic PAH and ascites by preserving right-heart function, suppressing pulmonary arterial remodeling, enhancing antioxidant capacity, and inhibiting CaSR signalling. In conclusion, salidroside mitigated PAH and ascites under high-altitude conditions by maintaining normal right heart function, reducing pulmonary artery pressure, enhancing antioxidant capacity, and modulating CaSR expression.

INTRODUCTION

Pulmonary arterial hypertension (PAH), also known as broiler ascites syndrome (AS), is characterized by the accumulation of pale-yellow fluid (50–400 mL) in the abdominal cavity in affected broilers, along with right heart failure, hypertrophy, and damage to vital organs such as the heart, liver, and lungs (Khajali & Wideman, 2016). Broilers raised in high altitudes are more susceptible to ascites, leading to high mortality rates. Ascites is primarily caused by chronic hypoxia, which elevates hematocrits and increases pulmonary vascular resistance, leading to elevated pulmonary arterial pressure and right ventricular hypertrophy. Such CaSR-mediated mechanism concurs with field observations: AA broilers reared at 2,990 m in Linzhi, Xizang exhibit a 14 % mortality attributable to ascites that clusters with the same risk factors of hypoxia induction—ambient oxygen insufficiency, high nutritional-demand diets, micronutrient deficiencies, toxicity and coinfection—, suggesting hypoxia as the unifying factor (Bourgeois *et al.*, 2020; Shlosberg *et al.*, 1998).

The calcium-sensing receptor (CaSR), which is a member of the family C of the G protein–coupled receptors (Abe *et al.*, 1999), is the main sensor of extracellular calcium and therefore a guardian of calcium homeostasis (Cheshmedzhieva *et al.*, 2021). In pulmonary arterial smooth muscle cells (PASMCs), activated CaSR raises cytoplasmic Ca^{2+} levels (Zhou *et al.*, 2021); this enhances PASMCs proliferation and consequently the vascular remodeling that is a symptom of PAH (Yu *et al.*, 2022). Thus, by regulating intracellular Ca^{2+}, CaSR directs both growth of the smooth-muscle and thickening of the pulmonary vascular wall.

Rhodiola rosea, a high-altitude (4,000–5,100 m) species that withstands wide diurnal temperature swings, is traditionally used to combat altitude sickness. Its primary glycoside, salidroside, exerts antioxidant, anti-fatigue, antitumor, and immunomodulatory effects, protecting the heart, brain, liver, lung and other organs from hypoxic injury (Chen *et al.*, 2012; Qu *et al.*, 2012; Wang *et al.*, 2013). We therefore hypothesized that dietary salidroside would prevent hypoxia-induced pulmonary arterial hypertension and ascites in broilers. To test this, we reared AA broilers at 2,990 m (Linzhi, Xizang) for 42 d in hypoxic (14–16 % O_2), normoxic (20.8–21.5 % O_2), or hypoxic + salidroside (50 mg kg^{-1} feed) conditions and assessed mortality, hemodynamics, antioxidant status, vascular histology and CaSR expression. The findings clarify the CaSR-dependent mechanism by which salidroside mitigates pulmonary vascular remodeling and provide a molecular basis for using this botanical extract to safeguard poultry health in high-altitude production systems.

MATERIALS AND METHODS

Experimental Animals

All broilers were housed in the Veterinary Building of the Xizang Institute of Agriculture and Animal Husbandry (2,990 m above sea level), and randomly assigned to three experimental groups with four replicates per group (15 birds per replicate). The groups were as follows: ① Hypoxia group (H): exposed to the natural high-altitude hypoxia environment at the housing site (ambient oxygen concentration: 14.0–16.0 % O_2); ② Normoxia group (N): maintained under standard oxygen levels (20.8–21.5 % O_2) through the use of an oxygen generator, following the method described by Wang Lihong (Wang, 2018); ③ Salidroside group (S): reared under the same hypoxic conditions as group H, with 0.2 % salidroside powder added to the basal diet. In addition to the above conditions, each group received the same daily ration of feed and water at the same feeding times. On the 42nd day of the feeding trial, 5 mL of blood was collected from the wing vein of each broiler using sterile needles and syringes. The blood samples were centrifuged at 2,800 rpm for 15 min to separate serum, and the resulting serum was stored at –20 °C for subsequent determination of antioxidant indices and immunoglobulin contents.

Determination of Right Heart Index and Mortality Rate of Broilers

Following euthanasia, the entire heart was excised. Excess adipose tissue was carefully removed, and the left atrium, right atrium, and associated large vessels were dissected away to isolate the total ventricular mass (TV). The right ventricular mass (RV) was then separated, and the right ventricular hypertrophy index (RV/TV) was calculated as the ratio of RV to TV. Mortality was recorded daily, with all deceased birds subjected to necropsy. Chickens were classified as having ascites-related mortality if they presented abdominal hydrops, hydropericardium, vascular occlusion, or an RV/TV ratio > 0.29; all other deaths were attributed to non-ascites causes.

Determination of the Serum Antioxidant Indices and Immunoglobulin Levels in Broilers

After 42 days of feeding, 5 mL of blood was collected from the wing vein of chickens in each of the three groups, and serum was subsequently separated. Following the manufacturers' instructions, the concentrations of four serum antioxidant indices were determined by ELISA, using commercial assay kits from Wuhan Elerit Biotechnology Co., Ltd. The indices and their corresponding detection methods were as follows: superoxide dismutase (SOD, hydroxylamine method), malondialdehyde (MDA, TBA method), glutathione peroxidase (GSH-Px), and catalase (CAT).

Immunohistochemistry

After a 42-day feeding regimen, pulmonary artery tissue was collected. The tissue was embedded in paraffin, sectioned, and the sections were incubated with 3% H_2O_2 solution at room temperature in the dark for 30 min. Antigen retrieval was performed using a repair buffer, followed by blocking each slide

with 50 µL of 3% TBST solution. The sections were incubated with rabbit anti-CASR antibody (1:200; Wuhan Elerit Biotechnology Co., Ltd.), then with goat anti-rabbit HRP-conjugated IgG antibody (Wuhan Elerit Biotechnology Co., Ltd.). DAB was applied for chromogenic development, and the sections were counterstained with hematoxylin for 2 min, dehydrated through a graded alcohol series, cleared, and mounted. Negative controls were prepared by substituting PBS buffer for the primary antibody. Observations and image acquisition were conducted using an Olympus optical microscope.

RT-qPCR

Total RNA was extracted from each sample using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. cDNA was synthesized by reverse transcription using the Genecopoeia All-in-One™ First-Strand cDNA Synthesis Kit (Genecopoeia, Inc., USA). Real-time quantitative PCR (RT-qPCR) was performed using 2× All-in-One™ qPCR Mix (Genecopoeia, Inc., USA) in a 20 µL reaction system, which contained 10 µL of qPCR mix, 8 µL of nuclease-free water, 1 µL of cDNA template, and 0.5 µL of each primer. The thermal cycling program consisted of initial denaturation at 95 °C for 10 min, followed by 38 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s. Primer sequences are listed in Table 1.

Table 1 – RT-qPCR primer information.

Genens	Primer Sequences (5'→3')	Product length(bp)	Annealing temperature (°C)TM
CaSR	F:CCACTCACCTTTGTGCTGTC R:GGCACTGGCATCTGTCTCA	165	60
β-*actin*	F:CTGTGCCCATCTATGAAGGCTA	139	59

Western-Blot

Proteins from chicken pulmonary artery tissue were extracted and quantified using the bicinchoninic acid (BCA) assay. An aliquot containing 20 µg of protein was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane. After blocking with 5 % skim milk at room temperature for 2 h, the membranes were incubated overnight at 4 °C with primary antibodies—rabbit anti-CaSR polyclonal antibody (Abcam, ab137408) at 1:10,000 and rabbit monoclonal anti-GAPDH antibody [EPR16891] (Abcam, ab181602) at 1:2,000—followed by HRP-conjugated goat anti-rabbit IgG (Abcam, ab6721) at 1:5,000 for 1 h at room temperature. The membranes were washed three times (5 min each) with Tris-buffered saline containing 0.1% Tween-20 (TBST). Immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) detection kit, and band intensities were quantified with the ImageJ software. Relative target-protein levels were expressed as the ratio of CASR to GAPDH band intensities.

Statistical Analysis

Statistical analyses were conducted using one-way analysis of variance (ANOVA) in SPSS version 26.0, followed by least significant difference (LSD) *post hoc* tests. Data are presented as mean ± standard deviation (SD), and statistical significance was set at $p<0.05$ unless otherwise stated.

RESULTS

Right Heart Index and Chicken Mortality in Each Group

The average mortality rates and right heart indices of the three broiler groups at day 42 are presented in Table 2. Groups S and N exhibited considerably lower right heart indices (0.25 and 0.24, respectively) than Group H (0.32, $p<0.05$). Group N had the lowest mortality rate (13.33%), followed by Group S (15%), while Group H showed the highest rate (21.67%), which was substantially greater ($p<0.05$). Ascites-related mortality was also considerably lower in Groups N (5%) and S (6.67%) than in Group H (10%, $p<0.05$). However, no significant differences were detected in either the right heart index or overall mortality between Groups S and N ($p>0.05$).

Table 2 – Right heart index and broiler mortality in each treatment group.

Groups	Right heart index (RV/TV)	Ascites mortality (%)	Non-ascites mortality (%)	Total mortality (%)
Hypoxic (h)	0.32 ± 0.04^{b}	10.00^{b}	11.67^{b}	21.67^{b}
Normal (n)	0.24 ± 0.03^{a}	5.00^{a}	8.33^{a}	13.33^{a}
Salidroside (s)	0.25 ± 0.02^{a}	6.67^{a}	8.33^{a}	15.00^{a}

Note: Different lowercase letters indicate a significant difference ($p<0.05$) as compared to the same index, while the same letter does not indicate a significant difference ($p>0.05$).

Serum Antioxidant Indices of Chickens in Each Group

The serum antioxidant indices of the three broiler groups are presented in Table 3. Serum SOD and GSH-Px activities in Groups S and N were significantly higher than those in Group H ($p<0.05$), whereas MDA levels were significantly lower ($p<0.05$). CAT activity

in Group N was substantially higher than in Group H (p<0.05), with no significant difference observed between Groups N and S (p>0.05).

Table 3 – Broiler serum antioxidant indices in each treatment group.

Group	SOD (U/mL)	MDA (nmol/L)	GSH-Px (μmol/L)	CAT (U/mL)
Hypoxic (h)	167.62±13.52^{a}	4.68±0.52^{b}	323.63±34.22^{a}	3.27±0.92^{a}
Normal (n)	189.61±16.70^{b}	3.36±0.33^{a}	411.26±43.71^{b}	4.09±0.55^{b}
Salidroside (s)	185.13±14.34^{b}	3.53±0.70^{a}	398.83±44.46^{b}	3.62±0.37^{ab}

Note: Different lowercase letters indicate a significant difference (p<0.05) as compared to the same index, while the same letter does not indicate a significant difference (p>0.05).

Histopathological Changes in Pulmonary Artery Tissue of Broilers

Hematoxylin and eosin (H&E) staining was performed on the pulmonary artery tissues of broilers. As shown in Figure 1, the pulmonary artery wall of the normoxic group (Panel A) exhibits an intact structure, with a clearly defined endothelium and no evidence of abnormal thickening or inflammatory cell infiltration, indicating a physiologically normal vascular state. In contrast, the hypoxic group (Panel B) displays marked thickening of the vascular wall, accompanied by smooth muscle cell proliferation and inflammatory cell infiltration. These alterations indicate that, relative to the normoxic group, the pulmonary artery underwent pathological remodeling under hypoxic conditions.

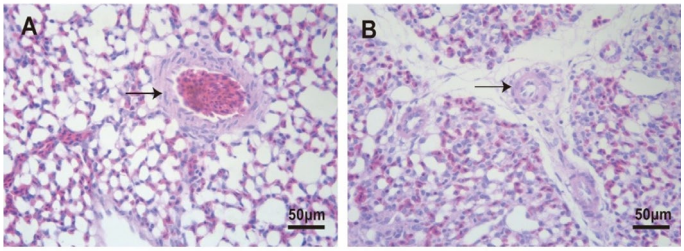

Figure 1 – Histopathological evaluation of pulmonary artery tissues in broilers from control and hypoxic groups (H&E staining, ×400).

Expression of Proliferation and Apoptosis-Related Markers in Pulmonary Artery Tissue

To further assess the effects of normoxia and hypoxia on vascular cell proliferation and apoptosis, immunohistochemical staining for caspase-3 (CAS3), Ki67, and proliferating cell nuclear antigen (PCNA) was performed on pulmonary artery tissues from broilers. Significant differences in CAS3, Ki67, and PCNA expression were observed between the normoxic and hypoxic groups. As shown in Figure 2, CAS3 expression was markedly reduced in the hypoxic group, as evidenced by a weaker brown-yellow positive signal, indicating a significant decrease in apoptosis within pulmonary arterioles. Conversely, expression levels of the proliferation-related proteins Ki67 and PCNA were significantly elevated under hypoxic conditions, indicating enhanced proliferative activity. These findings suggest that hypoxia promotes proliferation while inhibiting apoptosis in endothelial and smooth muscle cells of the pulmonary artery, which demonstrates that hypoxic conditions significantly enhance cellular proliferation and inhibit apoptosis within the vascular wall of pulmonary arteries.

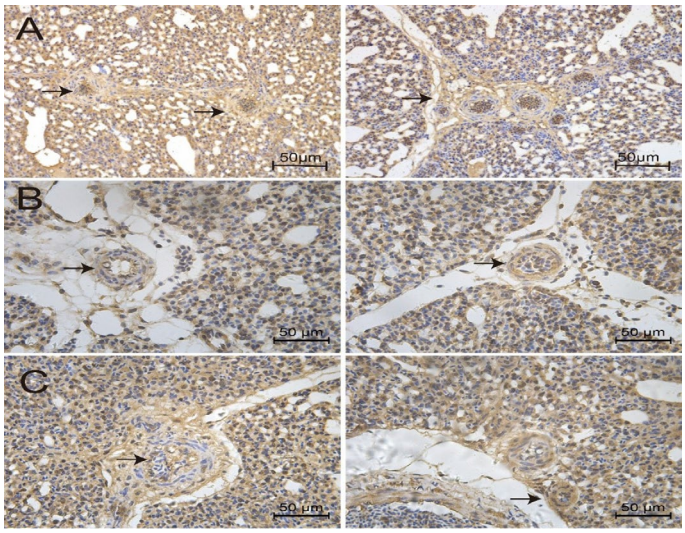

Figure 2 – Expression of Caspase-3 (CAS3), Ki67, and PCNA proteins in the normoxic (left) and hypoxic groups (right).
Note: A: Caspase-3 (CAS3); B: Ki67; C: PCNA.

Distribution of CaSR Protein in Broiler Pulmonary Artery Tissues

Figure 3 shows the distribution of CaSR protein in the smooth muscle layer of the pulmonary artery in broilers from different treatment groups. In the negative control (Figure 3A), no specific CaSR staining was observed, and cell nuclei appeared blue–purple. In the treatment groups, varying intensities of tan cytoplasmic staining indicated CaSR protein expression, with nuclei remaining blue–purple. Group S (Figure 3D) and Group N (Figure 3C) exhibited weaker tan staining than Group H (Figure 3B), indicating lower CaSR protein expression (staining intensity positively correlates with expression level). These findings suggest that salidroside supplementation under hypoxic conditions reduces CaSR protein expression in the pulmonary arteries of broilers.

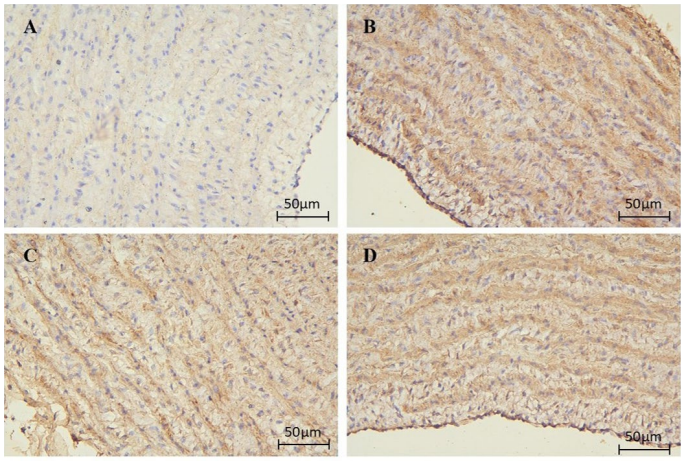

Figure 3 – Distribution of CASR protein in the smooth muscle tissue of the pulmonary artery of broilers (400×).
A: Negative control; B: Hypoxia group; C: Normal oxygen group; D: Salidroside group.

Relative Expression of CaSR mRNA in Pulmonary Artery Tissues of Chickens in Each Group

As shown in Figure 4, the relative expression of CaSR mRNA in the smooth muscle layer of the pulmonary artery was significantly lower in Groups S and N than in Group H (p<0.05), with no significant difference observed for Groups S and N (p>0.05).

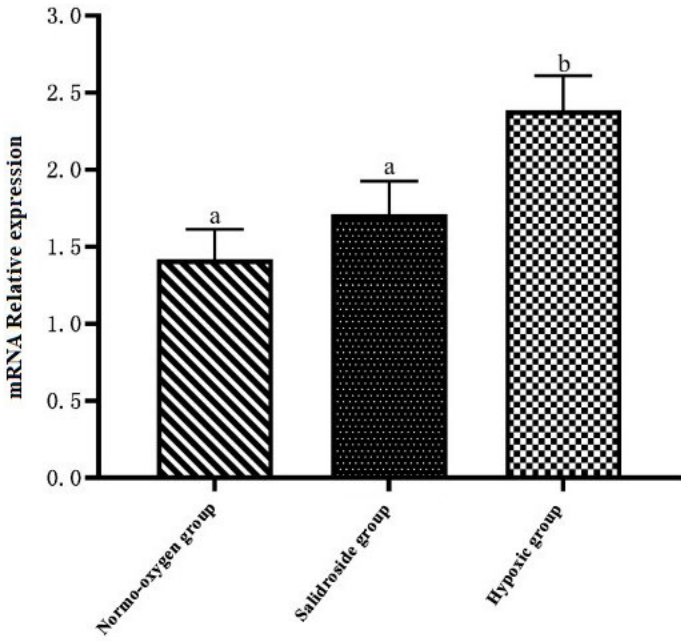

Figure 4 – Differential expression of CaSR mRNA in the smooth muscle tissue of the pulmonary artery.

CaSR Protein Expression in the Pulmonary Arteries of Chickens in Each Group

As shown in Figure 5, CaSR protein expression in the pulmonary arteries of the three groups followed the same trend as mRNA expression, with significantly lower levels in Groups S and N than in Group H (p<0.05).

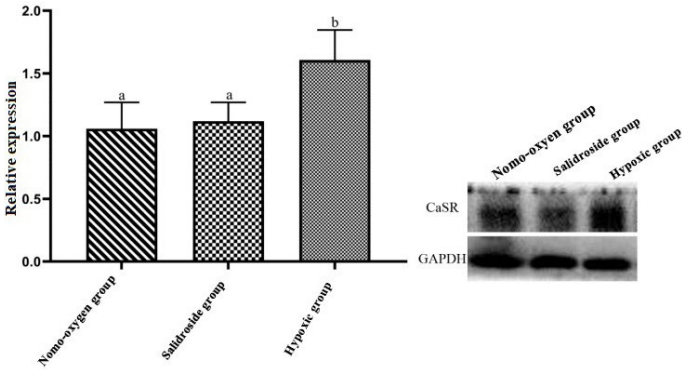

Figure 5 – Expression of the CaSR protein in the pulmonary artery smooth muscle tissue of broilers.

DISCUSSION

AA broilers are characterized by high growth rates and metabolic demands, which create an exceptionally high oxygen requirement. However, the development of their cardiorespiratory systems often lags behind, potentially leading to chronic hypoxia and the subsequent development of PAH. In these broilers, chronic high-altitude hypoxia aggravates tissue hypoxia and precipitates ascites. Salidroside—a bioactive component previously proven to mitigate oxidative stress and inhibit apoptosis—was discovered to relieve pulmonary vascular remodeling and enhance cardiovascular function, thus precluding PAH and ascites (Zhang *et al.*, 2018). Our findings in the current study showed that the ascites-induced mortality was much lower compared to the hypoxia group but was not significantly different from the normoxic group, a sign of the efficacy of salidroside in the prevention of ascites under hypoxic stress. This protection could be a result of its ability to relieve pulmonary hypertension induced by vascular remodeling, hence restoring physiological parameters to a level comparable to normoxic conditions (Yang *et al.*, 2012). Our results also showed that, after 42 days of feeding, both total mortality and non-ascites-related mortality were significantly lower in the salidroside-supplemented and normoxic groups than in the hypoxic group. Notably, hypoxia-induced physiological impairment weakened the birds' disease resistance and increased their susceptibility to infection. On the other hand, normoxia maintains normal physiological processes and maintains high disease resistance, which results in a low rate of mortality (Tymchik *et al.*, 2021). In its turn, salidroside enhances antioxidant capacity, strengthens immune function, and improves broiler adaptability to

hypoxic environments, thereby reducing mortality (Wu *et al.*, 2022).

In this investigation, initial histological and immunohistochemical studies compared the normoxic and hypoxic groups to investigate the pathological underpinnings of hypoxia-induced vascular damage. Our analyses of H&E staining showed that hypoxia drastically thickened pulmonary arterial walls and stimulated smooth muscle cell growth, which are evidences of vascular remodeling. Consistent with these observations, our immunohistochemical studies demonstrated a marked reduction in caspase-3 (CAS3) expression in hypoxic pulmonary arterioles, suggesting reduced apoptosis. In contrast, our studies showed that expression of the proliferating markers Ki67 and proliferating cell nuclear antigen (PCNA) was markedly elevated, suggesting enhanced cellular proliferation in hypoxia. These findings suggest that hypoxia disturbs the homeostasis between proliferation and apoptosis in vascular tissues, thereby causing pulmonary vascular remodeling.

In subsequent experiments, inclusion of a salidroside-treated group provided early evidence that this compound exerts vascular protective effects, potentially through modulating expression of CaSR and thus minimizing hypoxia-evoked vascular remodeling. Our follow-up experiment data provide a theoretical basis for further investigation into salidroside's protective role.

Wherever PAH happens, elevated pulmonary artery pressure causes the right ventricle to do more work, decreasing cardiac output and increasing residual volume of blood, ultimately leading to right ventricular hypertrophy (Stickel *et al.*, 2019). Right ventricular index (RV/TV, that is, right ventricular mass to total ventricular mass ratio) has significant diagnostic value for PAH in broilers, and values greater than 0.29 suggest pulmonary hypertension (Thenappan *et al.*, 2018). For instance, in studies using a CaSR inhibitor in myocardial hypertrophy models, it was observed that suppressing CaSR expression could reduce ventricular hypertrophy through the reduction of cardiomyocyte apoptosis (Baghbanzadeh & Decuypere, 2008). In the present study, our findings revealed that the average RV/TV value of the hypoxia group on day 42 was 0.32, which is above the diagnostic cut-off level for pulmonary hypertension (>0.29) (Ding *et al.*, 2022). This finding indicates that hypoxia-induced vascular remodeling and pulmonary hypertension were paired with elevated RV/TV values. In contrast, the salidroside and normoxic groups had average RV/TV values of 0.25 and 0.24, respectively, both significantly lower than the hypoxia group. This suggests that salidroside effectively reduced RV/TV to levels comparable to normoxia, thereby avoiding PAH in broilers.

During aerobic respiration or exogenous stress, the body produces free radicals. The radicals are ordinarily neutralized by antioxidant enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), which convert toxic peroxides to non-toxic hydroxyl compounds and thereby maintain redox homeostasis (Bouayed & Bohn, 2010). The activity of the enzymes reflects the capacity of the organism to scavenge reactive oxygen species (ROS). However, when ROS production exceeds physiological or antioxidant enzyme activity becomes limiting, excess ROS will result in oxidative damage to biological macromolecules. This type of damage results in the accumulation of malondialdehyde (MDA), a biomarker for membrane lipid peroxidation. Elevated MDA levels indicate higher membrane damage and can be utilized as an indirect assay for aging and stress tolerance in animals and plants (Wideman *et al.*, 2000). Specifically, higher MDA content indicates greater lipid peroxidation and reduced antioxidant capability.

In rapidly growing broilers, which are under high oxygen demands, studies have associated chronic high-altitude hypoxia exposure with a higher incidence and mortality of ascites syndrome (AS) compared with low altitude conditions. Studies have identified disruption of free radical metabolism under hypoxia as one of the determinants of AS development (Fleming, 2008). In the present study, broilers reared under hypoxic conditions exhibited the lowest serum antioxidant indices (SOD, GSH-Px, and catalase [CAT]) among the three groups, suggesting a marked decline in antioxidant capacity during adaptation to hypoxia. Specifically, the salidroside treatment group showed distinctly elevated activities of GSH-Px and SOD and decreased MDA content compared with the hypoxia group ($p<0.05$), but without significant difference from the normoxic group ($p>0.05$). These findings indicate that salidroside might prevent or reduce the hypoxia-induced decrease in serum antioxidant capacity in broilers and thus maintain antioxidant status close to normal. We therefore predict that salidroside elevates serum antioxidant capacity during hypoxia, thus partially reversing its adverse effects.

To elucidate the molecular basis by which salidroside attenuates hypoxia-induced pulmonary artery remodelling, we focused on the calcium-sensing receptor (CaSR) signaling axis. CaSR, a class-C

G-protein-coupled receptor that governs systemic Ca^{2+} homeostasis, also orchestrates cell proliferation, differentiation, ion-channel gating, and hormone secretion (Kinoshita, 2017). As the primary regulator of intracellular calcium ([Ca^{2+}]cyt), CaSR dysfunction or mutation disrupts ion metabolism. PAH—defined as chronically elevated pulmonary arterial pressure—develops from chronic vascular constriction and remodeling. Interestingly, elevated [Ca^{2+}]cyt in PASMCs is a critical stimulus for vasoconstriction (Song *et al.*, 2014). In idiopathic PAH, studies have shown that increased expression of CaSR is a requirement for the proliferation of PASMCs and [Ca^{2+}]cyt elevation. Therefore, high Ca^{2+} expression in pulmonary arterial smooth muscle promotes [Ca^{2+}]cyt elevation and cell proliferation, thereby causing vascular remodeling, vasoconstriction, and hypertension (Zhang *et al.*, 2024). In the long term, this mechanism leads to arterial stenosis (AS) in broilers, supporting the "calcium ion excitation" hypothesis. Studies confirm that hypoxia activates CaSR, inducing PASMCs proliferation via the G-protein–PLC–IP3 pathway and vascular remodeling (Kuhr *et al.*, 2012; Brown, 2013; Lee *et al.*, 2019). Current studies also demonstrate that hypoxia-induced upregulation of CaSR in pulmonary arteries promotes PASMCs proliferation and vascular remodeling by enhancing intracellular calcium levels, which ultimately leads to PAH. In this study, we investigated CaSR mRNA and protein expression in pulmonary artery tissues of three broiler groups, focusing on the effects of 0.2% salidroside supplementation (Rodriguez *et al.*, 2020). Our results showed a significant reduction in CaSR mRNA and protein expression in the salidroside group compared with the hypoxia group, suggesting that high-altitude hypoxia upregulates CaSR in pulmonary arterial smooth muscle, while salidroside suppresses this upregulation. Consistent with previous research, the salidroside group had lower overall and ascites-related mortality than the hypoxia group, and the right heart index remained within non-PAH limits (Deng *et al.*, 2021). These findings indicate that salidroside lowers [Ca^{2+}] cyt in PASMCs, inhibits cell proliferation and vascular remodeling, maintains normal pulmonary artery pressure, and prevents ascites in broilers under hypoxia, thereby reducing mortality at high altitudes.

CONCLUSION

Our results demonstrated that salidroside suppressed the expression of CaSR in plateau broiler pulmonary artery tissue, suggesting that it inhibits [Ca^{2+}] cyt in PASMCs, inhibits cell proliferation, and alleviates pulmonary vascular remodeling. Our results also suggested that salidroside maintained the right heart index and pulmonary artery pressure at normal levels and thereby inhibited the onset of PAH in high-altitude broilers. Our results further showed that it encouraged resistance and adaptability, reduced the incidence of disease, and offered an innovative method for prevention of ascites syndrome in broilers by enhancing serum antioxidant capacity and IgG and IgA levels.

ACKNOWLEDGEMENTS

We sincerely thank the College of Animal Science and the Veterinary Clinical Laboratory of Tibet Agricultural and Animal Husbandry University for providing equipment support, and express our gratitude to the reviewers and editors for their hard work and professional contributions, which have helped improve the quality of this manuscript.

AUTHOR CONTRIBUTIONS

Writing - preparation of original draft, review and editing, YRY, ZQZ and SK; acquisition of funds, ZYC. All authors have read and agreed to the published version of the manuscript.

FUNDING

This study was supported by the National Key Research and Development Program (2022YFD1600902-3) and The Key Subject (XK2024-02). Science and Technology Programme of Linzhi City, Tibet Autonomous Region [grant number XDHZ-2025-07].

DATA AVAILABILITY STATEMENT

Data will be made available on request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abe J, Suzuki H, Notoya M, *et al.* Ig-hepta, a novel member of the G protein-coupled hepta-helical receptor (GPCR) family that has immunoglobulin-like repeats in a long N-terminal extracellular domain

and defines a new subfamily of GPCRs. Journal of Biological Chemistry 1999;274(28):19957–64. https://doi.org/10.1074/jbc.274.28.19957

Baghbanzadeh A, Decuypere E. Ascites syndrome in broilers: physiological and nutritional perspectives. Avian Pathology 2008;37(2):117–26. https://doi.org/10.1080/03079450801902062

Bouayed J, Bohn T. Exogenous antioxidants—double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative Medicine and Cellular Longevity 2010;3(4):228–237. https://doi.org/10.4161/oxim.3.4.12858

Bourgeois A, Lemay SE, Grobs Y, *et al.* A new cornerstone in the regulation of both vascular remodeling and right ventricle failure in pulmonary arterial hypertension. Circulation 2020;142(Suppl 3).

Brown EM. Role of the calcium-sensing receptor in extracellular calcium homeostasis. Best Practice & Research Clinical Endocrinology & Metabolism 2013;27(3):333–43. https://doi.org/10.1016/j.beem.2013.02.006

Chen SF, Tsai HJ, Hung TH, *et al.* Salidroside improves behavioral and histological outcomes and reduces apoptosis via PI3K/Akt signaling after experimental traumatic brain injury. PLoS One 2012;7(9):e45763. https://doi.org/10.1371/journal.pone.0045763

Cheshmedzhieva D, Ilieva S, Permyakov EA, *et al.* Ca^{2+}/Sr^{2+} selectivity in calcium-sensing receptor (CaSR): implications for strontium's anti-osteoporosis effect. Biomolecules 2021;11(11):1576. https://doi.org/10.3390/biom11111576

Deng L, Chen J, Wang T, *et al.* PDGF/MEK/ERK axis represses Ca^{2+} clearance via decreasing the abundance of plasma membrane Ca^{2+} pump PMCA4 in pulmonary arterial smooth muscle cells. American Journal of Physiology-Cell Physiology 2021;320(1):C66–C79. https://doi.org/10.1152/ajpcell.00290.2020

Ding Y, Hu Y, Yao X, *et al.* Dietary essential oils improve the growth performance, antioxidant properties and intestinal permeability by inhibiting bacterial proliferation and altering the gut microbiota of yellow-feather broilers. Poultry Science 2022;101(11):102087. https://doi.org/10.1016/j.psj.2022.102087

Fleming RH. Nutritional factors affecting poultry bone health. Proceedings of the Nutrition Society 2008;67(2):177–83. https://doi.org/10.1017/S0029665108007015

Gupta AR. Ascites syndrome in poultry: a review. World's Poultry Science Journal 2011;67(3):457–68. https://doi.org/10.1017/S004393391100050X

Khajali F, Wideman RF. Nutritional approaches to ameliorate pulmonary hypertension in broiler chickens. Journal of Animal Physiology and Animal Nutrition 2016;100(1):3–14. https://doi.org/10.1111/jpn.12315

Kuhr FK, Smith KA, Song MY, *et al.* New mechanisms of pulmonary arterial hypertension: role of Ca^{2+} signaling. American Journal of Physiology-Heart and Circulatory Physiology 2012;302(8):H1546–H1562. https://doi.org/10.1152/ajpheart.00944.2011

Lee JJ, Liu X, O'Neill D, *et al.* Activation of the calcium sensing receptor attenuates TRPV6-dependent intestinal calcium absorption. JCI Insight 2019;4(5):e128013. https://doi.org/10.1172/jci.insight.128013

Li D, Fu Y, Zhang W, *et al.* Salidroside attenuates inflammatory responses by suppressing nuclear factor-κB and mitogen activated protein kinases activation in lipopolysaccharide-induced mastitis in mice. Inflammation Research 2013;62(1):9–15. https://doi.org/10.1007/s00011-012-0545-4

Liu W, Zhang Y, Lu L, *et al.* Expression and correlation of hypoxia-inducible factor-1α (HIF-1α) with pulmonary artery remodeling and right ventricular hypertrophy in experimental pulmonary embolism. Medical Science Monitor 2017;23:2083–2088. https://doi.org/10.12659/msm.900354

Qu ZQ, Zhou Y, Zeng YS, *et al.* Protective effects of a *Rhodiola crenulata* extract and salidroside on hippocampal neurogenesis against streptozotocin-induced neural injury in the rat. PLoS One 2012;7(1):e29641. https://doi.org/10.1371/journal.pone.0029641

Rodriguez M, Chen J, Jain PP, *et al.* Upregulation of calcium homeostasis modulators in contractile-to-proliferative phenotypical transition of pulmonary arterial smooth muscle cells. Frontiers in Physiology 2021;12:714785. https://doi.org/10.3389/fphys.2021.714785

Shlosberg A, Bellaiche M, Berman E, *et al.* Comparative effects of added sodium chloride, ammonium chloride, or potassium bicarbonate in the drinking water of broilers, and feed restriction, on the development of the ascites syndrome. Poultry Science 1998;77(9):1287–1296. https://doi.org/10.1093/ps/77.9.1287

Song S, Yamamura A, Yamamura H, *et al.* Flow shear stress enhances intracellular Ca^{2+} signaling in pulmonary artery smooth muscle cells from patients with pulmonary arterial hypertension. American Journal of Physiology-Cell Physiology 2014;307(4):C373–C383. https://doi.org/10.1152/ajpcell.00115.2014

Squires EJ, Julian RJ. The effect of dietary chloride and bicarbonate on blood pH, haematological variables, pulmonary hypertension and ascites in broiler chickens. British Poultry Science 2001;42(2):207–12. https://doi.org/10.1080/00071660120048465

Stickel S, Gin-Sing W, Wagenaar M, *et al.* The practical management of fluid retention in adults with right heart failure due to pulmonary arterial hypertension. European Heart Journal Supplements 2019;21(Suppl K):K46–K53. https://doi.org/10.1093/eurheartj/suz207

Thenappan T, Ormiston ML, Ryan JJ, *et al.* Pulmonary arterial hypertension: pathogenesis and clinical management. BMJ 2018;360:j5492. https://doi.org/10.1136/bmj.j5492

Tymchik S, Bryksin O, Omelyanchuk E. Influence of different types of loads on the cardiovascular and muscular systems in the process of physical education classes in the department of athletic gymnastics. Picture Coding Symposium 2021;0(5(136)):128-32.

Wang L. Effects of oxygen concentration and probiotics on broilers in high altitude areas and the study of probiotics alleviating salmonella enteritis in chicks [D]. Yangling (CN): Northwest A&F University; 2018.

Wang Y, Xu P, Wang Y, *et al.* The protection of salidroside of the heart against acute exhaustive injury and molecular mechanism in rat. Oxidative Medicine and Cellular Longevity. 2013;2013:507832. https://doi.org/10.1155/2013/507832

Wideman RF, Fedde MR, Tackett CD, *et al.* Cardio-pulmonary function in preascitic (hypoxemic) or normal broilers inhaling ambient air or 100 % oxygen. Poultry Science 2000;79(3):415-25. https://doi.org/10.1093/ps/79.3.415

Wu Y, Yang F, Jiang W, *et al.* Effects of compound probiotics on intestinal barrier function and caecum microbiota composition of broilers. Avian Pathology 2022;51(5):465-75. https://doi.org/10.1080/03079457.2022.2100740

Xian H, Zhao J, Zheng Y, *et al.* MADP, a salidroside analog, protects hippocampal neurons from glutamate induced apoptosis. Life Sciences 2014;103(1):34-40. https://doi.org/10.1016/j.lfs.2014.02.040

Yu W, Xu G, Chen H, *et al.* The substitution of SERCA2 redox cysteine 674 promotes pulmonary vascular remodeling by activating IRE1α/XBP1s pathway. Acta Pharmaceutica Sinica B 2022;12(5):2315-29. https://doi.org/10.1016/j.apsb.2021.12.025

Kinoshita Y. The functions of calcium-sensing receptor in regulating mineral metabolism. Clinical Calcium 2017;27(4):491-7.

Yang Y, Zhang BK, Liu D, *et al.* Sodium hydrosulfide prevents hypoxia-induced pulmonary arterial hypertension in broilers. British Poultry Science 2012;53(5):608-15. https://doi.org/10.1080/00071668.2012.728284

Zhang Y, Yao Y, Wang H, *et al.* Effects of salidroside on glioma formation and growth inhibition together with improvement of tumor microenvironment. Chinese Journal of Cancer Research 2013;25(5):520-6. https://doi.org/10.3978/j.issn.1000-9604.2013.10.01

Zhou MY, Cheng L, Chen L, *et al.* Calcium-sensing receptor in the development and treatment of pulmonary hypertension. Molecular Biology Reports. 2021;48(1):975-81. https://doi.org/10.1007/s11033-020-06065-3

Zhang JJ, Chen JH, Zhao MP, *et al.* The role of endoplasmic reticulum stress in pulmonary hypertension in rat induced by chronic hypoxia and hypercapnia. Chinese Journal of Applied Physiology. 2018;34(4):327-33.

Zhang J, Li Q, Liao P, *et al.* Calcium sensing receptor: a promising therapeutic target in pulmonary hypertension. Life Sciences. 2024;340:122472. https://doi.org/10.1016/j.lfs.2024.122472

DISCLAIMER/PUBLISHER'S NOTE