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# Therapeutic effects of Salidroside on broiler ascites syndrome via the PI3K/AKT/mTOR Pathway

# Efectos terapéuticos del salidrosido en el síndrome de ascitis de pollos de engorde a través de la vía PI3K/AKT/mTOR

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## ABSTRACT

This study aimed to examine the impact of salidroside on PI3K, AKT, and mTOR expression in the lung tissue of broilers afflicted with ascites syndrome, and to elucidate its potential mechanism against ascites syndrome. Ninety Qingjiao Ma chickens were randomly allocated to three groups of thirty each: the model, treatment, and control groups. To induce ascites syndrome, both the model and treatment groups were fed a basal diet enriched with high fat (5 % rapeseed oil), high protein (4 % fish meal), and high sodium (0.12 % NaCl in drinking water) from 1 day of age to 42 days of age. The treatment group additionally received 0.2 % salidroside. All the chickens were euthanatized at 42 days of age, and lung tissue was collected. The mRNA and protein expression levels of PI3K, AKT and mTOR in lung tissues was analysed by qRT-PCR, IHC, and WB, and one-way analysis of variance (one-way ANOVA) was used for statistical analysis with a significance level set at $P < 0.05$. The expression of PI3K, AKT, and mTOR - at mRNA and protein level was highly up-regulated in the ascites syndrome induced broiler when compared with control group ($P < 0.05$), including their phosphorylated form. The salidroside treatment reversed the changes by decreasing gene and protein level expressions of those signal molecules relative to the model group ($P < 0.05$). PI3K/AKT/mTOR signal transduction pathway is involved in the occurrence and development of ascites syndrome, salidroside may exert therapeutic effects on ascites syndrome byprobably by inhibiting the PI3K/AKT/mTOR pathway.

**Key words:** Broiler ascites syndrome; PI3K/AKT/mTOR pathway; salidroside

## RESUMEN

El presente estudio tuvo como objetivo examinar el impacto del salidrósido en la expresión de PI3K, AKT y mTOR en el tejido pulmonar de pollos de engorde afectados por el síndrome ascítico, así como dilucidar su posible mecanismo de acción contra esta patología. Noventa pollos Qingjiao Ma fueron asignados aleatoriamente a tres grupos de treinta individuos cada uno: grupo modelo, grupo de tratamiento y grupo control. Para inducir el síndrome ascítico, tanto el grupo modelo como el de tratamiento recibieron una dieta basal enriquecida con alto contenido de grasa (5 % de aceite de colza), alta proteína (4 % de harina de pescado) y alto sodio (0,12 % de NaCl en el agua de bebida) desde el día 1 de edad hasta los 42 días de edad. El grupo de tratamiento recibió adicionalmente un 0,2 % de salidrósido en su dieta. Todos los pollos fueron sacrificados a los 42 días de edad, y se recolectó tejido pulmonar para los análisis posteriores. Los niveles de expresión de ARNm y proteínas de PI3K, AKT y mTOR en los tejidos pulmonares se analizaron mediante técnicas de qRT-PCR, IHC y WB; para el análisis estadístico se aplicó el análisis de varianza de una vía (one-way ANOVA), estableciendo el nivel de significancia en $P < 0,05$. En comparación con el grupo control, la expresión de PI3K, AKT y mTOR —a nivel de ARNm y proteína, incluidas sus formas fosforiladas— se encontró muy regulada al alza en los pollos de engorde con síndrome ascítico inducido ($P < 0,05$). El tratamiento con salidrósido revertirá estos cambios al reducir las expresiones génica y proteica de dichas moléculas señalizadoras en relación con el grupo modelo ($P < 0,05$). La vía de transducción de señales PI3K/AKT/mTOR participa en la aparición y progresión del síndrome ascítico, y el salidrósido probablemente ejerce efectos terapéuticos sobre esta enfermedad mediante la inhibición de dicha vía de señalización.

**Palabras clave:** Síndrome de ascitis en pollos de engorde; vía PI3K/AKT/mTOR; salidrosido

## INTRODUCTION

Broiler (*Gallus gallus domesticus*) ascites syndrome (AS), which mainly occurs in modern,rapidly growing broiler chickens [1]. The primary pathologic event is hypoxic PH, which is defined as increased pulmonary artery pressure that places undue stress on the RV, andeventually resulting in right ventricular hypertrophy (RHV) and failure as well as transudation of fluid into the abdominal cavity [2].

In live animals, the clinical signs include a swollen abdomen, respiratory distress, and depression. In dead birds, there is ascites with a clear,pale yellow fluid (ascites) in the abdomen that may be associated with lesions of enlarged and congestive liver and pericardial effusion [3].

Ascites syndrome, which leads to stunted growth and high death rates among broiler chickens, has been identified as one of the top three nutrition/metabolism-related disease that are harmful for all poultry industries worldwide. Globally, AS affects 5–20 % of broiler flocks, with mortality rates of 1–8 % under commercial conditions and up to 30 % in severe cases, causing annual economic losses of approximately $1 billion USD [4]. Furthermore, AS exerts severe negative impacts on broiler production performance—including reduced body weight gain, decreased feed conversion efficiency, and lower marketable yields—underscoring its detrimental effects on both bird health and farm profitability [2].

The rising prevalence of AS is causing a huge loss to the poultry industry [5]. Rapid growth in broilers is considered a major contributing factor to the development of ascites. The increase in metabolic O2 demand necessary to support increased rates of muscular growth exceeds the capacity for cardiopulmonary system development, leading to insufficient functionality of the cardiopulmonary circulation [6].

Besides genetics, other contributing causes include environment (e.g. high altitude, poor barn ventilation), nutrition (e.g. high-energy/high-protein diet,shigh sodium drinking water), and management factors (e.g. high stocking density) may cause or worsen the disease [7, 8].

At present, prevention and treatment of broiler AS are still difficult. As there is not yet any breed of AS resistant broilers available, nutritional intervention and drug therapy are the two main modulating approaches [9, 10]. Nevertheless, the traditional drugs such as diuretics or vasodilators have poor effectiveness and adverse reactions. Thus, clarifying its pathogenesis as well as discovering new effective treatment methods is urgently needed [11, 12].

The pathogenesis of AS is extremely complicated. The current study mainly focuses on the oxidative stress and inflammation and autophagy [13]. The phosphoinositide 3-kinase / protein kinase B / mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway plays a pivotal role in regulating essential cellular functions such as proliferation, survival, metabolism, and angiogenesis, among others [14].

The PI3K/AKT/mTOR pathway is instrumental in driving key processes of PH, such as increasing Pulmonary vascular resistance (PVR), PVR index (PVRI) a mechanism linked to this signaling cascade [7]. right ventricular dysfunction, and metabolic reprogramming [15]. Activation of this pathway starts with PI3K,which promotes synthesis of the secondary messenger Phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [16].

Phosphorylated AKT activates several downstream targets such as the mTOR and forms an AKT- mTOR positive feedback loop that eventually controls protein synthesis,cell cycle progression, and glucose metabolism [17, 18]. The PI3K/AKT/mTOR pathway in normal physiological conditions is tightly regulated,maintaining the balance of pulmonary circulation .However, it is abnormally activated in response to stimuli like hypoxia, oxidative stress and autophagic dysregulation [19, 20]. This imbalance is a central player in the pathology of broiler AS, contributing critically to both its initiation and clinical deterioration [10].

Salidroside (SDS), a natural phytochemical, exhibits significant anti-proliferative and pro-autophagic effects in pulmonary vascular protection [21]. Recent in vitro evidence indicates that SDS mitigates aberrant pulmonary arterial smooth muscle cell (PASMC) proliferation through multiple mechanisms: 1) Suppression of PI3K/AKT pathway activation: SDS competitively binds to the PI3K catalytic domain, reducing the p-AKT/AKT protein expression ratio. This effectively impedes AKT-mediated signaling to mTOR, consequently inhibiting pathological cell proliferation [22]. 2) Downregulation of mTOR complex activity: By inhibiting phosphorylation of Raptor, a critical regulatory component of mTOR complex 1 (mTORC1), SDS reduces expression of phosphorylated p70S6K and 4E-BP1. This arrests cell cycle progression, ultimately suppressing aberrant PASMC proliferation [23].

The objective of this work was to analyse changes in the PI3K/AKT/mTOR signalisation pathway in lungs under broiler AS development,and determine whether SDS can regulate this pathway. Meanwhile, this study aimed to explore the effect of SDS treatment on AS in broilers,thus offering a scientific basis to unravel the mechanism of AS and develop new therapies.

## MATERIAL AND METHODS

### Experimental animal grouping

A total of ninety Qingjiao Ma chickens were randomly divided among three experimental groups (n = 30/group, see TABLE I): Model group,Treatment group,Control group. To induce AS, chickens in both the Model and Treatment groups were fed a basal diet that was enriched to be high in fat (5 % rapeseed oil), protein (4 % fish meal), and sodium (0.12 % NaCl provided in drinking water) from 1 day (d) of age to 42 days of age. Chickens assigned to the Treatment group additionally received 0.2 % SDS in their diet. On d 42, ten chickens per group were euthanized and lung tissues were collected for subsequent analyses.

**TABLE I**
***Experimental animal grouping and treatment protocol***

| Group | n | Treatment Protocol |
|---|---|---|
| Control | 30 | Standard basal diet |
| Model | 30 | High-fat/sodium diet-induced AS |
| Salidroside-treated | 30 | High-fat/sodium diet + 0.2 % salidroside supplementation |

Note: AS = Ascites syndrome; n = number of broilers per group.

## Ascites heartiIndex

The ascites heart index (AHI) is the ratio of right ventricular weight to total ventricular weight (RV/TV). At 42 d post-hatching, after blood sampling chickens were humanly killed by means of cervical dislocation followed by immediate removal of the heart as an intact organ to be studied. Samples were epicardial fat carefully excised. The heart chambers were subsequently flushed with physiological saline in order to remove blood clots without damaging tissues or compromising measurements. Atria and their great vessels were removed from the left side of the coronary sulcus. The remaining TV was weighed using an electronic analytical balance (Sartorius, CPA225D, Germany). The RV was then dissected from the interventricular sulci and weighed separately. Right ventricular hypertrophy (RVH) was quantified by calculating AHI = RV/TV.

## Detection of PI3K, AKT, and mTOR mRNA expression by RT-qPCR

Following collection, lung tissues were rinsed in physiological saline, flash-frozen in liquid nitrogen, and stored at -80°C (Meling, DW-HL780, China). Each sample approximately 100 mg powdering into the pre-chilled mortar on ice in liquid nitrogen continuously, total RNA was extracted via TRIzol Reagent.

The concentration and purity of the RNA were determined using a UV spectrophotometry (Thermo Fisher Scientific, NanoDrop 2000, USA). cDNA synthesis was performed with the PrimeScript RT reagent kit with gDNA Eraser (Sangon Biotech, KR126, China) following the manufacturer's protocol. Quantitative real-time PCR (qPCR) was conducted using FastReal FastFire qPCR PreMix (Tiangen, FP207, China) on a real-time PCR system (Bio-Rad, CFX opus 96, USA), with GAPDH serving as the reference gene. Gene-specific primers for PIK3CA, mTOR, AKT1, and GAPDH were commercially synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China), as specified in TABLE II.

The qPCR reaction system (20 μL) was composed of: cDNA (1 μL), forward primer (0.6 μL), reverse primer (0.6 μL), 2× Fast Real PreMix(10 μL), RNase-free ddH2O (7.8 μL). Amplification program: predenaturation at 95°C for 2 min;40 cycles of 95°C, 5 sec (denaturation); 60°C, 10 sec (annealing); 72°C, 15 sec (extension). Relative gene expression was calculated by the 2^(-ΔΔCt) method (TABLE II).

**TABLE II**
***Primer sequences used in qPCR analysis***

| Target Gene | Primer Sequence (5′→3′) |
|---|---|
| PIK3CA | F:AGGGTGCTAAAGAGGAGCACT<br>R:TCCATGGGGTACTGCCCAAA |
| mTOR | F:CACAACCACTGCTCGCCACAA<br>R:CCATAGGATCGCCACACGGATTAG |
| AKT1 | F:CACGCTGACAGAAAACCGTG<br>R:CAGCCCCATAAAAACGTGCC |
| GAPDH | F:GAAGGCTGGGGCTCATCTG<br>R:CAGTTGGTGGTGCACGATG |

## Immunohistochemical detection of PI3K, AKT, and mTOR protein expression

Lung tissue samples were fixed in 4 % paraformaldehyde (48 h) and then thoroughly rinsed. They were then processed through a graded ethanol series for dehydration, xylene for clearing, and ultimately embedded in paraffin. Sections (5 μm thickness) were cut using a rotary microtome (Leica, RM2255, Germany) and deparaffinized to water through standard protocols. Immunohistochemistry (IHC) was performed using a Streptavidin-Peroxidase (SP) Kit (Solarbio, SP0041, China) according to manufacturer's instructions. Primary antibody dilutions: anti-PI3K (1:100), anti-AKT (1:100), anti-mTOR (1:1000). After cover slipping, slides were scanned with an automated digital slide scanner (Unic, PRECICE 500B, China) for quantitative analysis.

## Western blot analysis

Protein extraction from lung tissue was performed using a lysis buffer supplemented with both protease and phosphatase inhibitors. Subsequently, the protein samples were resolved by SDS-PAGE (Sinsage, P2012, China) and then electrophoretically transferred onto Immobilon-P PVDF membranes (Bio-Rad, 1620177, USA) with a Trans-Blot Turbo transfer system (Bio-Rad, MiniVE, USA).

Following transfer, the membranes were blocked in 5 % BSA for 2 hours at room temperature, followed by an overnight incubation at 4 °C with specific primary antibodies. These included: Recombinant Anti-Phospho-AKT (Servicebio, S473, China) at 1:1000 dilution, Anti-Phospho-PI3K (Beyotime, AF5905, China) at 1:500, and Anti-Phospho-mTOR (Servicebio, S2448, China) at 1:500. β-Actin (Servicebio, GB11001, China) was applied for loading control.

Next, the membranes were incubated with an HRP-conjugated goat anti-rabbit IgG secondary antibody (Thermo Fisher Scientific, #A27036, USA) at a dilution of 1:10,000 for 1 h at room temperature. After three washes in TBST (Servicebio, G0004, China), the immunoreactive bands were visualized using ECL Substrate (Servicebio, G2161, China) and captured on X-ray film.

### Statistical analysis

Statistical analyses were performed using Excel (Microsoft). Graphs were generated with GraphPad Prism 10 (GraphPad Sof tware). Significance was determined by one-way ANOVA. Significance levels were defined as follows: ns (not significant, $P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), **** ($P < 0.0001$).

## RESULTS AND DISCUSSION

### Clinical signs and post-mortem of broiler ascites syndrome and salidroside's ameliorative effect

Broilers in the model group showed severe AS clinical signs (lethargy, closed eyes, reduced appetite, reluctance to move, ruffled dull feathers, pronounced abdominal distention, cyanotic tense shiny abdominal skin) and ventral recumbency or lameness. Salidroside-treated broilers had improved mental alertness, feather gloss, and reduced abdominal distention. Post-mortem examination confirmed massive clear pale-yellow ascitic fluid and pericardial effusion in the model group (FIG. 1).

These manifestations are typical of broiler AS [24]. Salidroside's improvement of clinical symptoms and reduction of ascitic fluid preliminarily confirm its therapeutic potential, consistent with its pleiotropic properties (anti-inflammatory, antioxidant, hemodynamic modulation) [25]. Unlike current therapies that only alleviate symptoms [24], SDS may target key AS pathological processes.

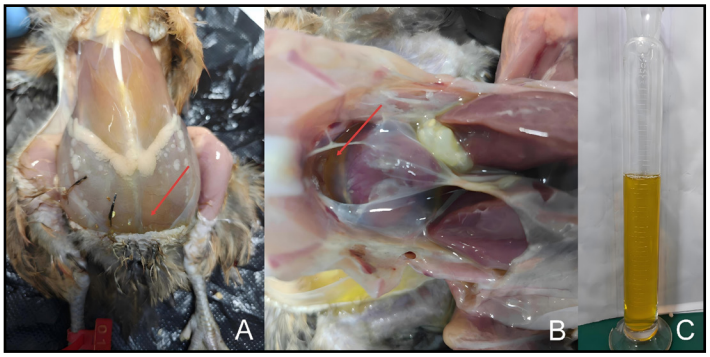


**FIGURE 1.** Gross Pathological Changes in broilers of the ascites syndrome (AS) model Group. (A) Abdominal cavity of the model broiler, revealing grossly distended visceral organs from accumulated ascites (red arrow points to tense, distended abdomen). (B) Dissected thorax of the model broiler, showing pronounced pericardial effusion around the heart (red arrow showing fluid filled pericardial sac). C straw coloured (pale yellow) ascites obtained from abdominal cavity of the model broiler and placed in a measuring cylinder for visualising amount and colour.

### Salidroside's effect on right ventricular hypertrophy in AS broilers

Right ventricle to total ventricle weight ratio RV/TV is a critical index for pulmonary hypertension syndrome (PHS) (RV/TV > 0.25 = RVH; > 0.29 = right heart failure (RHF), and persistent RHF is a major manifestation of severe heart failure (HF). As shown in TABLE III, AHI was significantly elevated in model and salidroside-treated group vs. controls ($P < 0.001$). Most importantly, SDS treatment resulted in a marked 13.2 % reduction in AHI (0.297 ± 0.03) compared to the model group (0.342 ± 0.05) ($P < 0.05$), demonstrating its efficacy in alleviating right ventricular afterload—a critical determinant of AS-related mortality [26].

The elevated AHI in the model group confirms successful AS induction, while the significant decrease following SDS intervention represents direct attenuation of right ventricular remodeling. This 13.2 % reduction is clinically meaningful, as previous studies have demonstrated that even modest RV/TV reductions (10-15 %) are associated with significantly improved survival outcomes in AS models [26]. By reducing AHI from 0.342 (severe RVH) to 0.297 (moderate RVH), SDS moves affected birds closer to the survival threshold (< 0.28), potentially explaining the improved clinical outcomes observed.

The therapeutic effect observed aligns with previous findings that SDS attenuates pulmonary arterial pressure in hypoxic broilers [26] and suppresses cardiac hypertrophy via PI3K/AKT/mTOR inhibition [22]. Unlike growth restriction or diuretic therapy—which only manage symptoms without reversing cardiopulmonary pathophysiology [11]—SDS leverages its pleiotropic mechanisms to target the root pathological processes, underscoring its distinct therapeutic advantage.

**TABLE III**
***Ascites heart index across experimental groups***

| Parameter | Control | Model | Model+SDS |
|---|---|---|---|
| AHI | $0.207 \pm 0.04^{A}$ | $0.342 \pm 0.05^{Ba}$ | $0.297 \pm 0.03^{Bb}$ |

Note: Alphabetical superscripts are used to mark statistical groupings. Identical lower-case letters (a, b) are assigned to bars with no significant difference ($P > 0.05$). Different lower-case letters (a, b) designate a significant difference ($P < 0.05$), and different upper-case letters (A, B) designate a highly significant difference ($P < 0.01$), as assessed using one-way ANOVA followed by Tukeys multiple comparison posttest. SDS: Salidroside. AHI: Ascites heart index.

### Effect of Salidroside on Growth Performance in Ascites Syndrome Broilers

Although this study focused on molecular mechanisms, the observed cardiopulmonary improvements should be translated into enhanced productivity. Previous studies confirm that dietary SDS (500 mg/kg) significantly increases average daily gain ($P < 0.01$) and improves feed conversion efficiency in broilers [27]. Rhodiola extract—rich in SDS—also enhances growth performance, with combination therapy showing superior efficacy [28].

Additionally, SDS improves meat quality by reducing oxidative markers (MDA, TBARS), decreasing drip loss, and enhancing antioxidant enzyme activities (SOD, GSH-Px) [29]. These findings support that the reduced AHI and PI3K/AKT/mTOR inhibition observed in our study would likely confer meaningful productivity benefits under commercial conditions.

## Salidroside Regulates the Expression of PI3K/AKT/mTOR Pathway mRNAs

RT-qPCR (FIG. 2): the expression of PI3K, AKT and mTOR mRNA was much more highly expressed in model group or SDS treated group than control ($P < 0.001$) ;the expression of PI3K, AKT and mTOR mRNA decreased significantly after SDS treatment compared with model group ($P < 0.001$), but it is still higher than that of control (PIK3CA, mTOR: $P < 0.001$; AKT1: $P < 0.05$). The upregulation of mRNAs in the model group confirmed that abnormal activation of PI3K/AKT/mTOR pathway exists in AS and leads to its pathology dysregulated cell proliferation [30], metabolic reprogramming [31], pulmonary vascular remodeling, right ventricular dysfunction [32]. SDS downregulates pathway activation on a transcriptional level but preserves basal activity, without undesirable side-effects of full suppression.

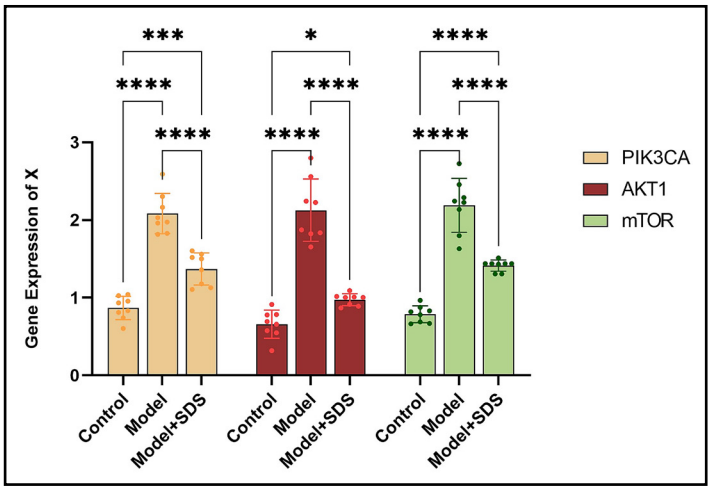


**FIGURE 2.** Salidroside (SDS) modulates mRNA expression of PIK3CA, AKT1 and mTOR in lung tissue. PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, AKT1: RAC-alpha serine/threonine-protein kinase 1, mTOR: Mechanistic target of rapamycin.

## Effect of Salidroside on the Protein Expression and Location of PI3K/AKT/mTOR Pathway

Immunohistochemical (FIG. 3) showed PI3K, AKT, and mTOR overexpression in alveolar epithelial cells, macrophages, fibroblasts, and myofibroblasts. Their protein levels were significantly elevated in model and salidroside-treated groups vs. control ($P < 0.01$), and SDS treatment significantly attenuated upregulation vs. model group ($P < 0.05$).

The localization of proteins on the important cells related to AS proves that these pathways are activated during the disease process. The decrease of these proteins by SDS also suggests a specific action, consistent with its antifibrotic effect (blocking profibrotic pathways, inhibiting collagen accumulation [33]. This finding corroborates the RT-qPCR data, showing that SDS regulates the pathway at both transcriptional and translational levels.

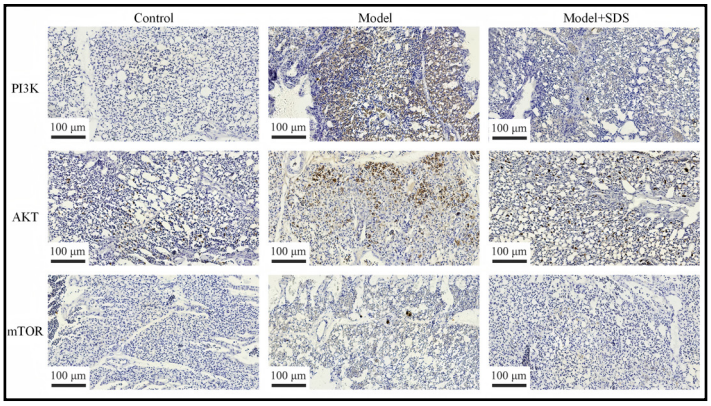


**FIGURE 3.** Immunolocalization of PI3K, AKT and mTOR proteins in lung tissues across
Note: PI3K: Phosphatidylinositol 3-kinase. AKT: Protein Kinase B. mTOR: Mechanistic Target of Rapamycin. SDS: Salidroside.

## Expression of P-PI3K, P-AKT, and P-mTOR proteins in lung tissue

Western blot (FIG. 4) showed elevated PI3K/AKT/mTOR in model group vs. controls ($P < 0.0001$); SDS intervention significantly reduced their levels vs. model group (p-PI3K, p-AKT: $P < 0.001$; p-mTOR: $P < 0.0001$).

Elevated phosphorylated proteins confirm pathway activation in AS. SDS inhibits pathway activation by competitively binding PI3K p110α catalytic domain, inhibiting AKT and mTORC1 activation [34], blocking pro-proliferative signaling and vascular remodeling. Combined with previous results, SDS mitigates AS via targeted suppression of the PI3K/AKT/mTOR pathway.

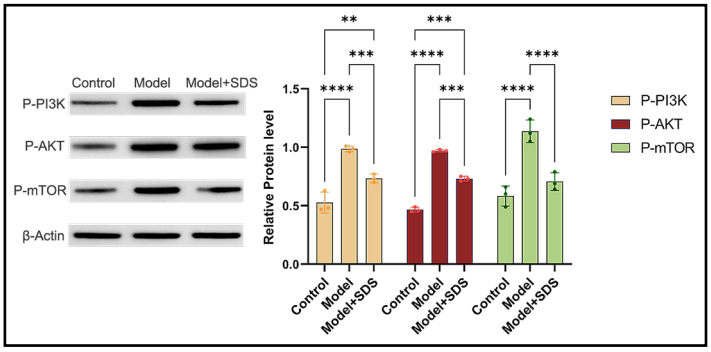


**FIGURE 4.** Expression of phosphorylated PI3K (P-PI3K), phosphorylated AKT (P-AKT) and phosphorylated mTOR (P-mTOR) proteins in lung tissues across different intervention groups. Left: Representative Western blot bands of P-PI3K, P-AKT, P-mTOR and internal reference β-Actin; Right: Quantitative analysis of relative protein levels (normalized to β-Actin).
Note: P-PI3K: phosphorylated phosphatidylinositol 3-kinase. P-AKT: phosphorylated Protein Kinase B. P-mTOR: phosphorylated Mechanistic Target of Rapamycin. β-Actin: Beta-Actin. SDS: Salidroside.

## CONCLUSION

The SDS can alleviate the pathological process of AS by inhibiting the PI3K/AKT/mTOR pathway, which provides an important theoretical basis for its potential clinical application. Adding 0.2 % SDS to the diet can reduce the outbreak incidence

rate of broiler ascites syndrome. Additionally, when broilers are at risk of ascites syndrome outbreak, SDS can be added to their diet to address the disease.

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### Ethical approval

The current study protocol was approved by Animal Ethics Committee of Tibet Agricultural and Animal Husbandry College (Approved number: XZA-2025-020).

### Conflicts of interest

The authors declare that there are no known conflicts of interest.

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