



## RESEARCH ARTICLE

### Baicalin Modulates CDH11/Wnt/ $\beta$ -catenin Pathway: A Novel Therapeutic Strategy Against Tibial Dyschondroplasia in Broiler Chickens

Kai Liu<sup>1,2</sup>, Khalid Mehmood<sup>3\*</sup>, Ying Li<sup>1</sup>, Yongxue Sun<sup>1</sup>, Aoyun Li<sup>4\*</sup> and Hui Zhang<sup>1,2\*</sup>

<sup>1</sup>College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China; <sup>2</sup>College of Animal Science, XiZang Agriculture and Animal Husbandry University, Linzhi 860000, China; <sup>3</sup>Faculty of Veterinary and Animal Sciences, Islamia University of Bahawalpur, Pakistan; <sup>4</sup>College of Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China

\*Corresponding author: khalid.mehmood@iub.edu.pk (Khalid Mehmood); aoyunli@henau.edu.cn (Aoyun Li); hz236@scau.edu.cn (Hui Zhang)

#### ARTICLE HISTORY (25-390)

Received: April 27, 2025  
Revised: July 01, 2025  
Accepted: July 13, 2025  
Published online: August 25, 2025

#### Key words:

Broiler chickens  
CDH11  
Chondrocyte differentiation  
Tibial dyschondroplasia  
Wnt/ $\beta$ -catenin

#### ABSTRACT

Tibial dyschondroplasia (TD) is a metabolic cartilage disorder in fast-growing broilers, which severely impacts poultry welfare and productivity. Baicalin is a bioactive flavonoid with anti-inflammatory and antioxidant properties; its therapeutic potential has not been studied for TD. This study paves the way in elucidating baicalin's chondroprotective mechanism through CDH11/Wnt/ $\beta$ -catenin signaling and evaluating its therapeutic efficacy. Notably, baicalin significantly improved TD broilers' symptoms (e.g., slow weight-gain, reduced feed intake) and up-regulated tibia morphometrics. Special staining showed abnormal cartilage enlargement in TD and self-healing groups, while histomorphometry indicated restored tibial trabecular architecture in treated groups. Mechanistically, the cartilage development-related genes CDH11, RUNX2, and alkaline phosphatase (ALP) were significantly up-regulated in the baicalin-treated group, and pivotal molecular factors in the Wnt/ $\beta$ -catenin-dependent signaling axis, which are involved in cartilage development, were up-regulated or down-regulated. These findings establish that baicalin mitigates TD pathogenesis through CDH11-dependent activation of canonical Wnt signaling, promoting chondrocyte maturation and skeletal remodeling, which will provide both mechanistic insights into avian skeletal metabolism and a clinically translatable strategy for metabolic bone diseases.

**To Cite This Article:** Liu K, Li A, Mehmood K, Li Y, Sun Y, and Zhang H 2025. Baicalin modulates CDH11/Wnt/ $\beta$ -catenin pathway: a novel therapeutic strategy against tibial dyschondroplasia in broiler chickens. Pak Vet J. <http://dx.doi.org/10.29261/pakvetj/2025.224>

#### INTRODUCTION

The skeletal system serves as the mechanical support core in vertebrates, with its homeostasis maintained through a dynamic equilibrium between osteoblast-mediated bone formation and osteoclast-regulated bone resorption (Li *et al.*, 2023). In avian biomechanical research, the tibia, a representative weight-bearing bone, exhibits a growth plate cartilage development pattern that serves as a critical biomarker for assessing skeletal health (Kianfar *et al.*, 2025). Morphogenesis of the tibial growth plate involves three key biological processes: programmed differentiation of chondrocytes, directional mineralization of the extracellular matrix, and spatiotemporal-specific vascular invasion (Osiak-Wicha *et al.*, 2025). Disruption of this regulatory network directly leads to Tibial dyschondroplasia (TD),

characterized by chondrocyte maturation arrest, impaired vascular infiltration, and abnormal cartilage plug formation (Shi *et al.*, 2024). Notably, under modern intensive farming, the incidence of TD in commercial broilers has risen significantly. Global epidemiological data indicate that TD accounts for 30% of all avian skeletal disorders, with prevalence rates exceeding 10% in large-scale poultry farms in China (Li and Hao, 2019; Zhang and Wang, 2020). At the pathomechanistic level, TD exhibits unique microenvironmental remodeling characteristics. Abnormal chondroprogenitor cell cycling: TD lesions tend to have an abnormal phenotype of "large cell-small cartilage capsule" (Wu *et al.*, 2024); TD-affected regions demonstrate a drastic reduction in vascularity (Sahin *et al.*, 2024); The boundaries between proliferative and hypertrophic areas in the TD growth plate disappear, and the arrangement of chondrocytes

shows disordered characteristics (Mehmood *et al.*, 2019). These pathological changes ultimately lead to characteristic motor dysfunction in TD broilers, including clinical signs such as progressive lameness, joint enlargement, and decreased feed intake.

Cadherin-11 (CDH11) plays specialized roles in skeletal development and homeostasis (Janczi *et al.*, 2025). Developmental studies reveal spatially restricted CDH11 expression in the embryonic chicken spinal cord, suggesting multifunctional roles in neural and paraspinal tissue morphogenesis (Manohar *et al.*, 2020). Mesenchymal Stem Cells (MSC) proliferation is mechanistically regulated via PDGFR-dependent signaling mechanisms, which are derived from platelet-derived growth factors (Passanha *et al.*, 2022). Concurrently, CDH11 exerts a transient modulation on the TGF- $\beta$  pathway to influence extracellular matrix (ECM) remodeling, and thereby steering MSC differentiation trajectories (Passanha *et al.*, 2022). Organogenesis, developmental patterning, and tissue homeostasis are governed by the Wnt/ $\beta$ -catenin signaling (Pham *et al.*, 2025). Notably, this pathway exerts indispensable control over osteoblastic differentiation and skeletal anabolism (Shen *et al.*, 2021). Canonical Wnt activation triggers  $\beta$ -catenin liberation from degradation complexes, facilitating its nuclear translocation and subsequent interaction with T-cell factor (TCF) transcription factors (Ferrando-Marc and Barkoulas, 2025). This molecular interplay culminates in the transcriptional activation of osteogenic targets, including RUNX2 (Molagoda *et al.*, 2021).

Baicalin, a key bioactive compound isolated from *Scutellaria baicalensis*, has been historically employed in traditional Chinese medicine for its therapeutic potential, particularly in exhibiting heat-clearing and detoxifying properties to address pathogenic conditions (Feng *et al.*, 2024). Mechanistically, baicalin modulates the RANK/RANKL/OPG pathway to rescue osteogenic impairment and enhance bone mineralization in zebrafish, while suppressing aberrant bone remodeling genes (Zhao *et al.*, 2020). This aligns with findings that baicalin promotes osteogenic differentiation while inhibiting osteoclastogenesis (Wang *et al.*, 2020). Notably, baicalin administration demonstrates therapeutic potential in avian models, effectively counteracting thiram-induced toxicity in broilers by preserving chondrocyte nuclear integrity, augmenting vascularization, and alleviating lameness (Iqbal *et al.*, 2023). Molecular analyses reveal its dual regulatory capacity: 1) upregulating osteoprotegerin mRNA expression through Wnt/ $\beta$ -catenin signaling activation, thereby stimulating osteoblast mineralization (Xiao *et al.*, 2023); 2) enhancing cellular viability, alkaline phosphatase (ALP) activity, and RUNX2 expression via coordinated activation of Wnt/ $\beta$ -catenin and MEK/ERK pathways.

However, the application of baicalin in avian bone lesions has rarely been reported. Therefore, this research was undertaken to assess the therapeutic potential of baicalin, focusing on its efficacy in alleviating clinical symptoms and mitigating pathological alterations, growth plate parameters in the context of injury in TD broiler chickens, and whether baicalin could be used as a therapeutic agent for TD. Genes and pathways related to

chondrocyte development were selected, and the effects of baicalin on TD in broilers were examined by their expression levels.

## MATERIALS AND METHODS

**Animal tria:** One hundred healthy, 1-day-old AA white-feathered broilers were selected and grouped, and they were fed as shown in Table 1. Thiram feed was 120 mg/kg of thiram powder added to the standard diet, and baicalein feed was 200 mg/kg of baicalein (90%) powder added to the standard diet and slaughtered on the 21st day. Experiments involving animals and experimental protocols were approved by the Institutional Animal Care and Use Committee at South China Agricultural University.

**Table 1:** Experimental grouping and treatment schedule

Groups	Treatment 1	Timeline (Day)	Treatment 2	Timeline (Day)	Treatment 3	Timeline (Day)
Group A	Normal feed	0-3	Normal feed	4-10	Normal feed	11-21
Group B	Normal feed	0-3	Thiram feed	4-10	Thiram feed	11-21
Group C	Normal feed	0-3	Thiram feed	4-10	Normal feed	11-21
Group D	Normal feed	0-3	Thiram feed	4-10	Baicalin feed	11-21

A: Control groups; B: Thiram groups; C: Thiram self-healing groups; D: Baicalin treatment groups.

**Histopathological analysis:** Tissue processing: Tibiae were decalcified in EDTA (30 d, solution refreshed weekly), dehydrated through graded ethanol (50%~100%), cleared in xylene, and paraffin-embedded.

**Sample size and control group treatment:** Each set of experiments consisted of three biological replicates (n=3). Negative controls were performed using PBS in place of primary antibodies, and positive controls were performed using sections of bone tissue known to be positive.

**H&E:** The sections were immersed in hematoxylin (10 min), then differentiated by 1% HCl, then immersed in eosin, and finally sealed with neutral gum.

**Toluidine blue:** The slices were stained for 3 minutes, using 0.1% glacial acetic acid for color separation, dried in an oven at 60°C, then soaked with xylene, and finally sealed with neutral gum.

**Alisin blue:** Staining was performed for 15~30 minutes, followed by staining with Nuclear Solid Red Staining Solution (G1035) for 3 minutes, followed by dehydration in anhydrous ethanol, xylene, and finally sealed with neutral gum.

**Solid green:** Sections were immersed in solid green staining solution for 1~5 minutes, quickly treated with 1% hydrochloric acid alcohol; then stained with senna red staining solution for 5~10 seconds, quickly dehydrated in anhydrous ethanol, transparent in xylene, and finally sealed with neutral gum.

**Goldner trichrome:** Mix Goldner's Stain A and Goldner's Stain B in equal volumes and immerse the sections for 20 minutes, then immerse them in Goldner's Stain C and D sequentially, decolorize twice with 0.2% glacial acetic acid, and immerse them in Goldner's Stain E for 3~5 minutes, and finally seal the sections with neutral gum.

**RT-qPCR:** Total RNA of tibial tissue was extracted by Trizol method (AG21102, AG) and using ABScript III RT Master Mix for qPCR with gDNA remover to complete cDNA synthesis. Amplification was performed using Genious 2× SYBR Green Fast qPCR Mix. Table 2 provides details of the primers used in this study.

**Table 2:** Primer Sequence Information

Genes	Primer sequence (5'-3')	Accession No.
<i>CDH11</i>	(F) AACAGACCTTTGGAACCGCC	NM_001004371.1
	(R) CGGCACATTGGCATGGTAGT	
<i>β-catenin</i>	(F) CCTGGTGCTGACTACCCAGT	NC_052533.1
	(R) CTCAGCAACTCTACAGGCCAA	
<i>GAPDH</i>	(F) AGTCAACGGATTGGCCGTA	NC_052532.1
	(R) TTCCCGTTTCTCAGCCTTGAC	

**Western blot analysis:** Tissues were homogenized in RIPA+PMSF, and quantified by BCA. Take proteins (25 µg/lane) for 10% SDS-PAGE electrophoresis analysis, transfer the membrane, and block non-specific binding sites. Add primary antibody followed by HRP-conjugated secondary antibody. Visualize sequentially using DAB and quantified via ImageJ. Antibody details are in Table 3.

**Table 3:** List of antibodies used during the study.

Names	Company	Category	Conjugation	Molecular size
<i>CDH11</i>	ABClonal	Polyclonal	Unconjugated	110 kDa
<i>RUNX2</i>	ABClonal	Polyclonal	Unconjugated	60 kDa
<i>COL2a1</i>	ABClonal	Polyclonal	Unconjugated	141 kDa
<i>BMP2</i>	ABClonal	Polyclonal	Unconjugated	15 kDa
<i>ALP</i>	ABClonal	Polyclonal	Unconjugated	25-35 kDa
<i>Wnt4a</i>	ABClonal	Polyclonal	Unconjugated	46 kDa
<i>β-catenin</i>	ABClonal	Polyclonal	Unconjugated	100 kDa
<i>GSK-3β</i>	ABClonal	Polyclonal	Unconjugated	46 kDa

**Immunostaining:** Immunofluorescence: Sections underwent antigen retrieval (10 min), Triton X-100 permeabilization (30 min), blocking with 10% horse serum (1 h), primary antibody incubation (4°C, 16 h), and secondary antibodies (1 h, dark). Nuclei were counterstained with DAPI.

Immunohistochemistry: Sections were antigenically repaired and treated with hydrogen peroxide for 15 min. Add primary antibody (4°C, 14 h) followed by HRP-conjugated secondary antibody, DAB color development, hematoxylin nuclear staining, hydrochloric acid alcohol staining, and finally sealed with neutral resin.

**Statistical analysis:** Statistical analysis was performed using Prism GraphPad 9.0 (San Diego, CA, USA). Experimental data are shown as means±SEM. Two-tailed Student's t-test assessed significance between two groups, while one-way ANOVA with Tukey's test was used for multiple comparisons. Statistical significance was set at  $P < 0.05$ .

## RESULTS

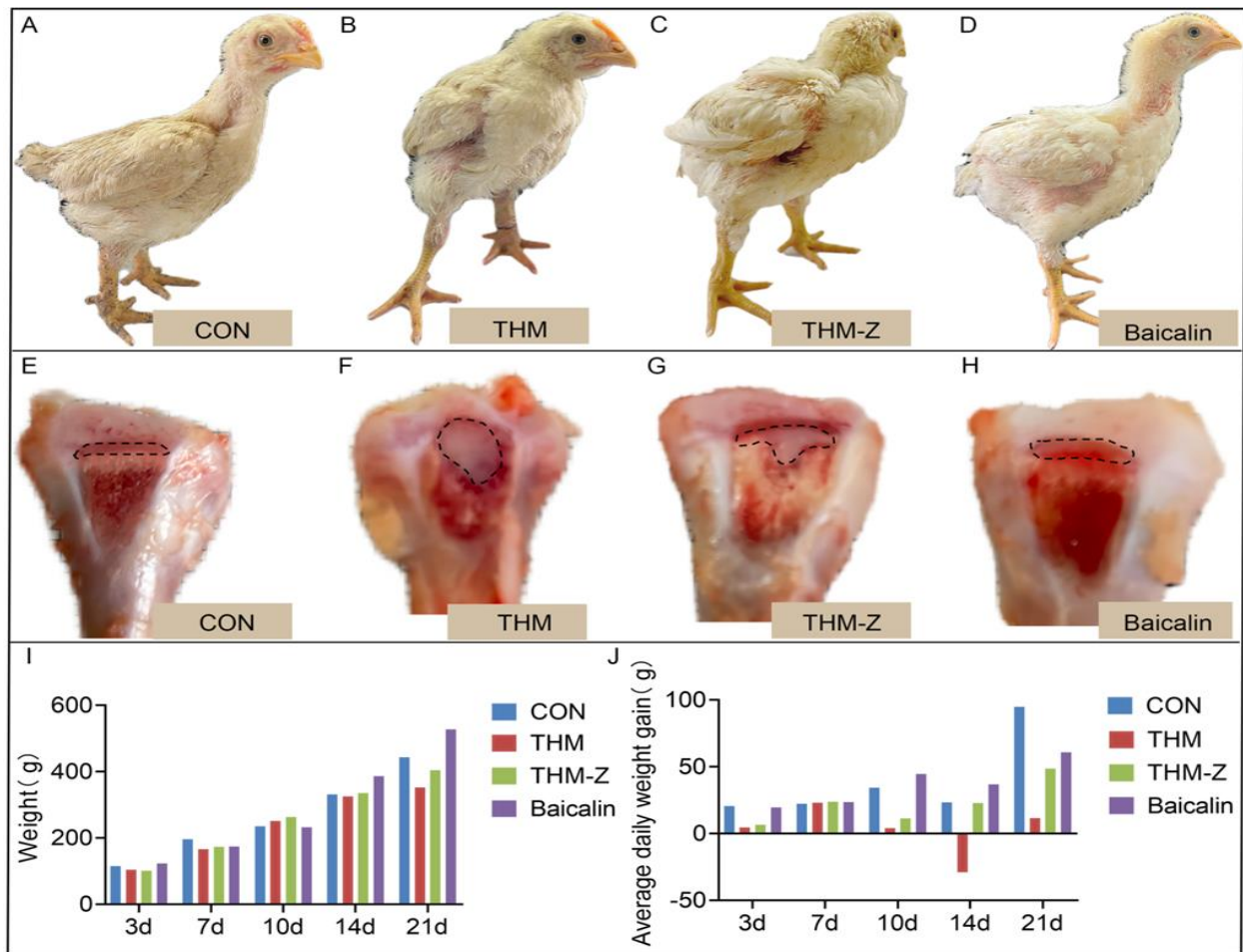
**Observations on clinical symptoms of TD broiler chickens and the effects of baicalin:** Following a 3-day adaptive feeding period, clinical observations have found that the control group of broiler chickens (Fig. 1A) were normal being active, while the TD group of broiler chickens (Fig. 1C) initially refused to feed and later experienced swelling of the leg joints, and in severe cases, a split posture. After replacing the feed, the TD self-healing

group broiler chickens (Fig. 1B) gradually began to eat and move independently. The broiler chickens in the baicalin treatment group (Fig. 1D) gradually began to eat and move independently after their feed was replaced. To further compare the developmental status of tibial growth plates, we randomly peeled the tibiae of different groups of broiler chickens. As shown in Fig. 1 E-H. An irregular, avascular, and uncalcified white cartilage thrombus was found in the growth plate of the TD group broiler chickens. The white cartilage thrombus area in the baicalin treatment group broiler chickens gradually decreased or tended to disappear, and there were obvious signs of vascular invasion in the growth plate. The results showed that in the first 7 days, the TD group, baicalin group, and TD self-healing group were all fed with thiram, and their body weight was lower (Fig. 1I). By day 14, the TD group showed a marked reduction in body weight relative to both the TD self-healing group and control animals. The baicalin treatment group showed significant weight gain. Analysis of the mean daily growth rate metrics revealed (Fig. 1J) that the TD group of broiler chickens showed slow growth or even negative growth, whereas the baicalin group of broiler chickens gradually increased in weight gain after 7 days of treatment.

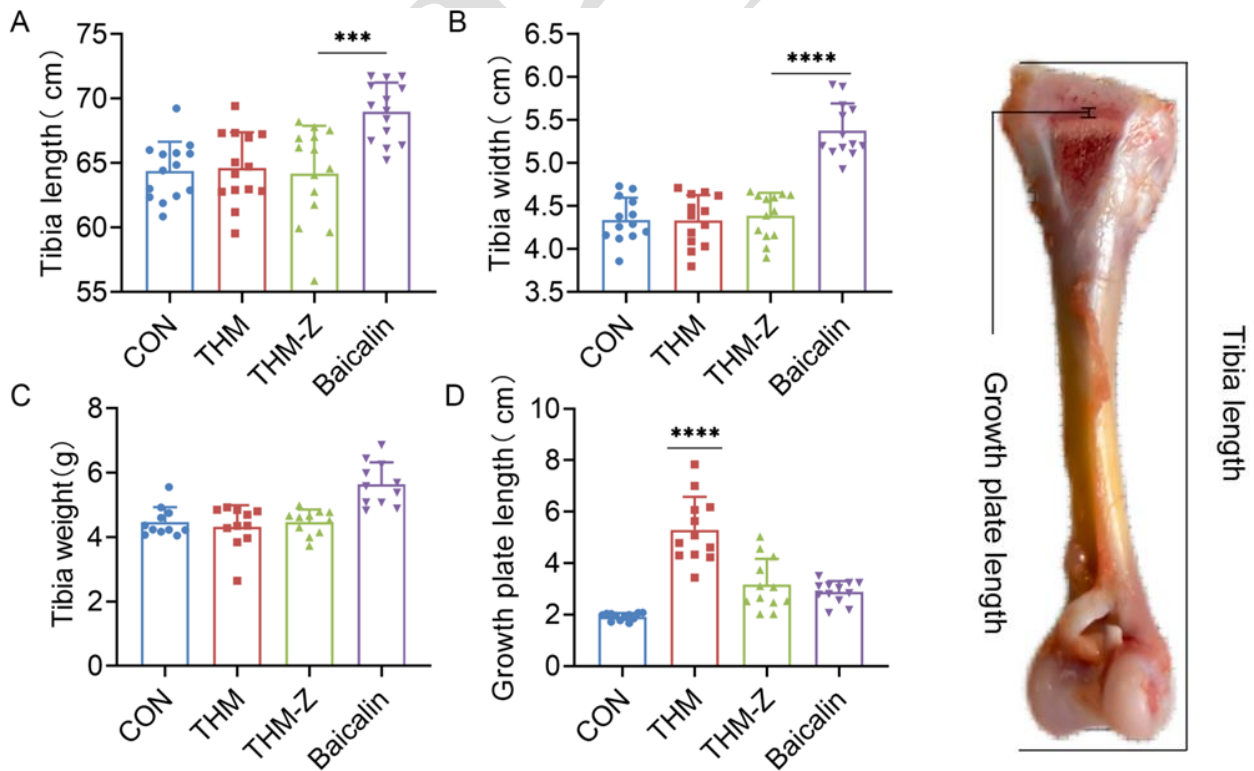
**The effect of baicalin on tibial development in broiler chickens:** The experiment found that the length, width, and weight of the tibia in the TD group (Fig. 2A-C) had no significant difference. Histomorphometric evaluation showed that the inner and outer spans of the proximal tibial growth zone in TD specimens were significantly larger ( $P < 0.0001$ ). Compared with TD and TD self-healing groups, the baicalin treatment group showed a significant increase in tibial-related indicators ( $P < 0.0001$ ). The baicalin intervention led to a notable reduction ( $P < 0.0001$ ) in the width of the tibial growth plate (Fig. 2D).

**H&E staining results of tibia tissue in broiler chickens:** To further understand the pathological changes in the tibial growth plate area after TD, one tibia in each group was randomly selected for tissue section H&E staining. The results are shown in Fig. 3. In the proliferation area and hypertrophic area of the growth plate under a 4× light microscope, the hypertrophic area was significantly larger, and the blood vessel distribution was significantly less, and the blood vessels were abnormal and blocked. In the baicalin treatment group, the ability of vascular invasion was restored, but the blood vessels were shorter, and the number of normal invasions to PZ was very small.

**Special staining results of tibia tissue in Broilers:** Goldner trichromatic staining showed that (Fig. 4A), tibial trabeculae in TD and TD self-healing groups were less, and they were thin and short, and arranged in disorder, while the amount of bone trabeculae in baicalin treatment group gradually increased, and gradually recovered to be strong and arranged regularly. Safranin fast green staining showed (Fig. 4B) that the chondrocytes in the control group were orderly arranged with clear boundaries and obvious signs of osteogenesis. The area of chondrocytes in the TD group significantly increased, while the baicalin treatment group showed a significant decrease in cartilage area, with significant osteogenesis. Alcian blue staining showed that

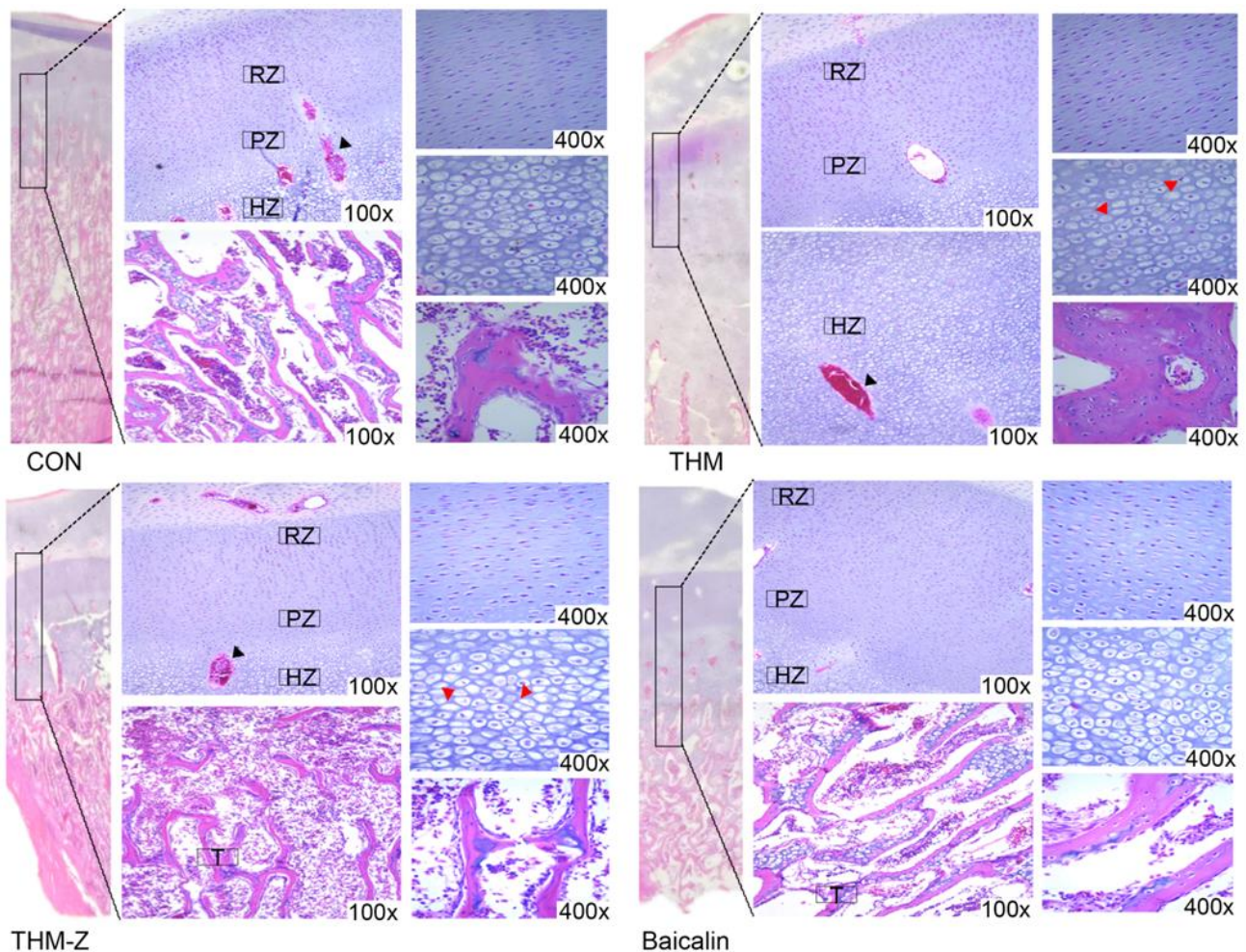


**Fig. 1:** Evaluation of growth status, tibial section and growth performance of broiler chickens. A-D) Clinical signs and differences in appearance of broiler chickens. E-H) Tibial growth plate development status. I) Broiler performance, body weight changes. J) Broiler performance, average daily weight gain.



**Fig. 2:** Tibial growth indicators. A) Tibial length. B) Tibial width. C) Tibial weight. D) Tibial growth plate length. One-way ANOVA with Tukey's test was used for multiple comparisons. Data are presented as the mean  $\pm$  SEM. n=12. \*\*\*P<0.001, \*\*\*\*P<0.0001.





**Fig. 3:** Histopathological observations on the tibial cartilage of broiler chickens. Note: RZ: resting zone; PZ: proliferative zone; HZ: hypertrophic zone; black triangles point to vascular areas, red triangles point to abnormal chondrocytes.

(Fig. 4C), the area of cartilage stained by alcian blue was significantly increased, while the area in the baicalin treatment group was decreased.

The results of toluidine blue staining (Fig. 5A-D) showed that cartilage and osteoblasts were blue-purple, with a light blue background. The arrangement of chondrocytes in the TD and TD self-healing groups was disordered. The chondrocytes treated with baicalin were arranged neatly, with obvious staining of the cell matrix and neat tide lines, without any tide line drift.

**Regulation of baicalin on the development of tibial cartilage:** The results of CDH11 and  $\beta$ -catenin (Fig. 6A-B) genes related to tibial cartilage development showed that a reduction expression level was observed, although the differences did not reach statistical significance ( $p > 0.05$ ); The expression of CDH11 and  $\beta$ -catenin (Fig. 6A-B) in the baicalin group was significantly upregulated ( $P < 0.001$ ). The expression results of proteins related to tibial cartilage development (Fig. 6C) showed that after treatment with baicalin, the expression of CDH11, RUNX2, ALP, and ACAN proteins increased. Among them, the expression of histone in ALP treated with baicalin was significantly ( $P < 0.05$ ) higher. Western blot analysis revealed alterations in the expression profiles of Wnt/ $\beta$ -catenin pathway constituents (Fig. 6D), the expression of TD histones Wnt4,  $\beta$ -catenin, and GSK-3 $\beta$  is

reduced. The TD self-healing group showed an increase, but lower than the control group, and significant upregulation of pathway-associated regulatory proteins was demonstrated in response to baicalin.

#### **Study on the regulatory effect of baicalin on proteins related to tibial cartilage development:**

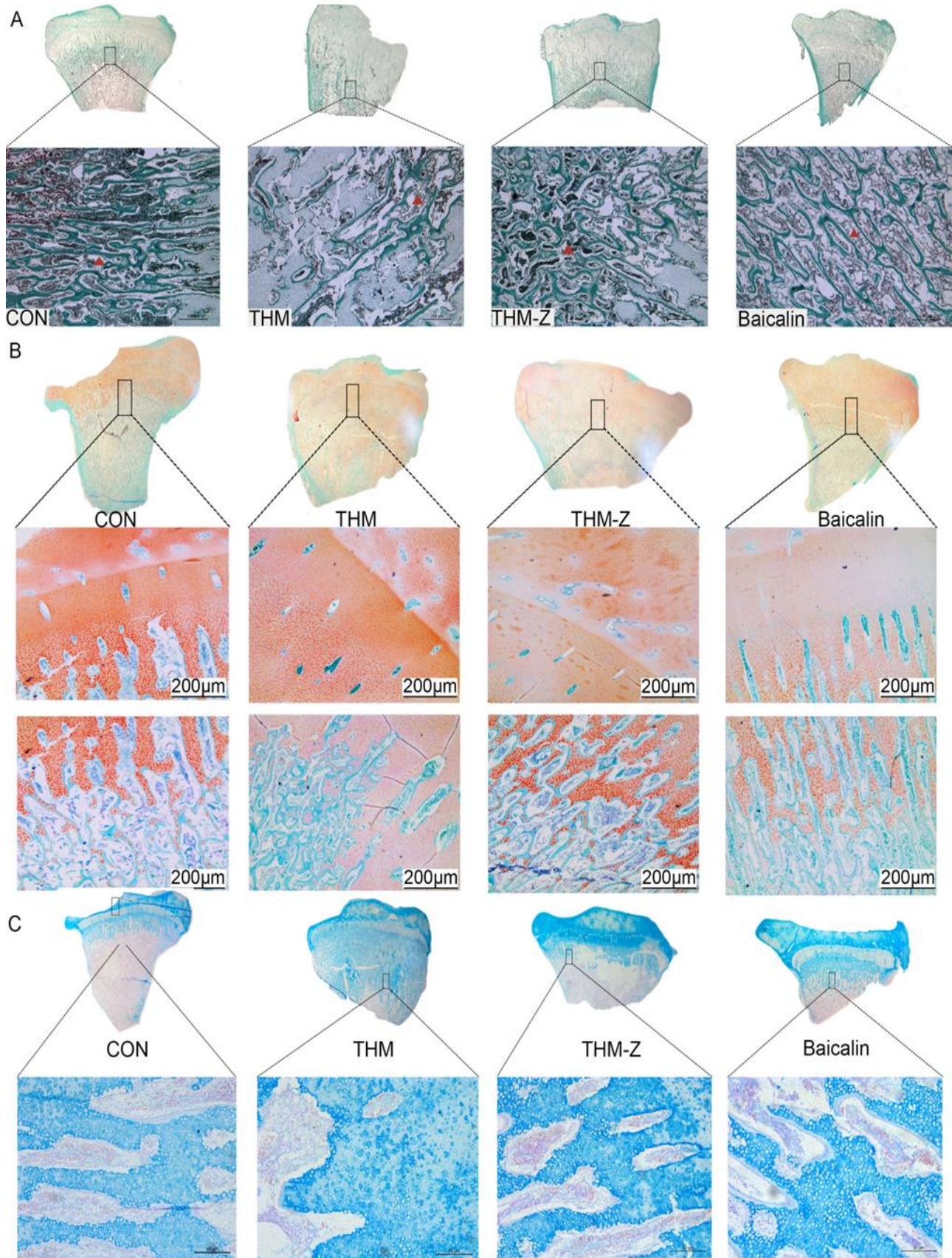
The localization results of baicalin on proteins related to the tibial cartilage tissue showed that CDH11, RUNX2, and BMP2 (Fig. 7A-C) showed decreased expression of TD histones. Baicalin administration induced a marked elevation in target protein expression levels ( $P < 0.001$  or  $P < 0.0001$ ). The TD histone expression of ALP and ACAN (Fig. 7D-E) decreased. The experimental group receiving baicalin administration demonstrated a substantial upregulation in target protein expression levels relative to the untreated TD self-healing group ( $P < 0.0001$ ).

The effect of baicalin on TD was demonstrated by immunofluorescence, and the TD group of CDH11 (Fig. 8A). A statistically significant elevation ( $P < 0.0001$ ) was observed in the baicalin-treated group compared to the TD self-healing group, which exhibited comparatively diminished values. RUNX2, BMP2, and ALP were associated with cartilage development (Fig. 8 B-D), the expression of histones decreased. A pronounced increase in histone expression was observed within the TD self-healing group subsequent to baicalin treatment ( $P < 0.0001$ ).



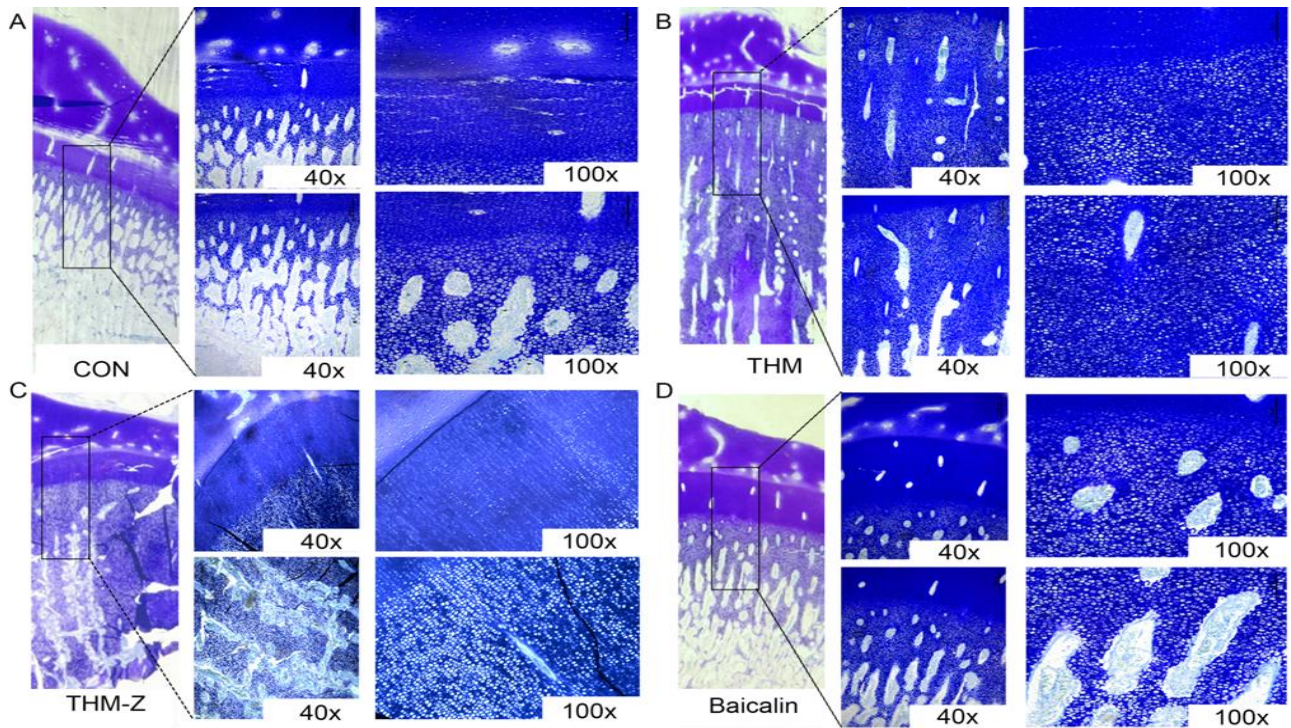
The TD histone expression of  $\beta$ -catenin (Fig. 8E) was reduced. However, the protein expression in the baicalin treatment group was significantly higher ( $p < 0.0001$ ).

GSK-3 $\beta$  (Fig. 8F), the expression of TD histones increased. The expression of histones after treatment with baicalin was significantly lower ( $P < 0.0001$ ).

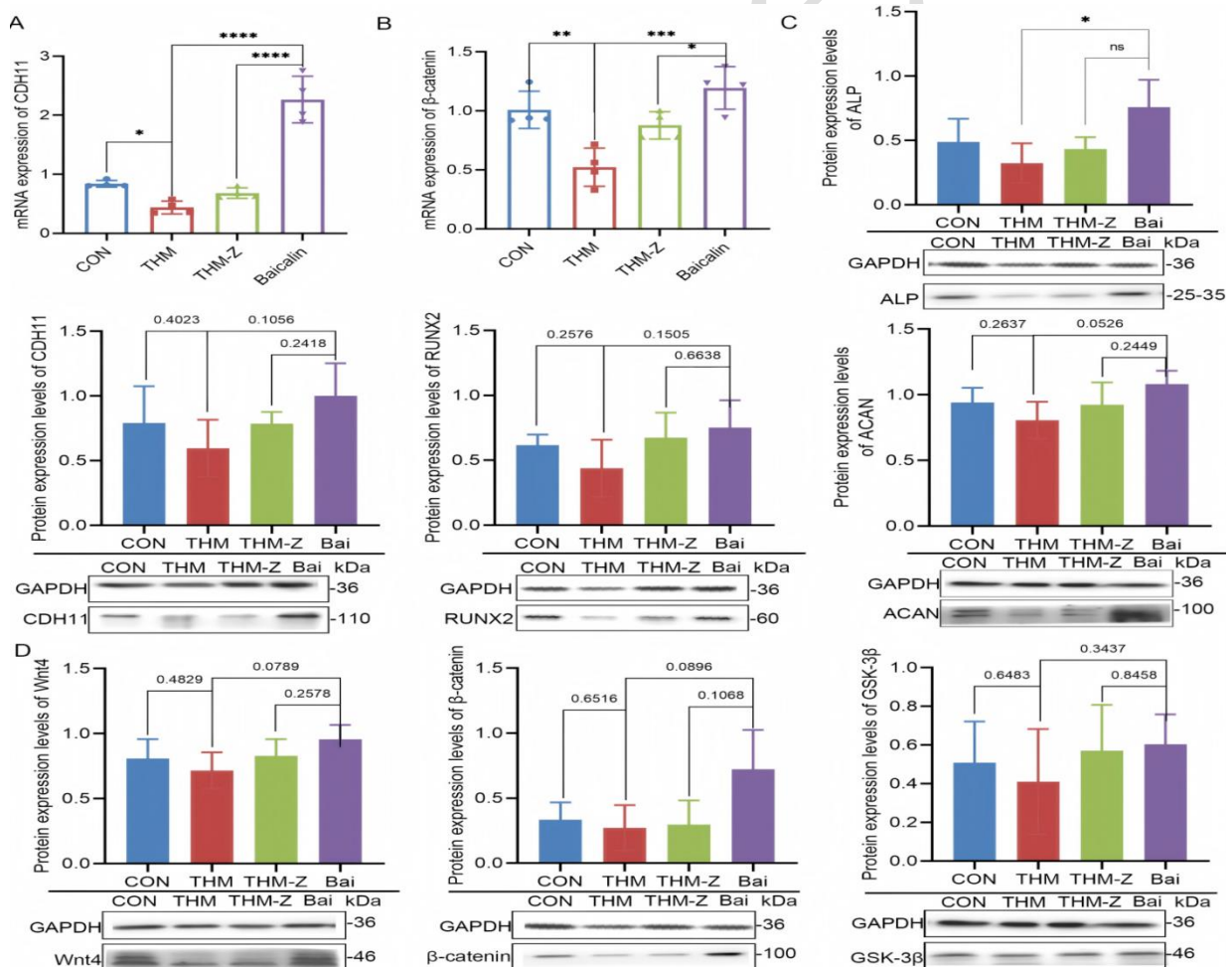


**Fig. 4:** Special staining of broiler tibia tissue. A) Tibial Tissue Goldner Stain. B) Tibia tissue stained with Senna- Solid Green. C) Alisin blue staining of tibial tissue. Note: Orange-red is non-mineralized bone (osteoid); green portion is mineralized bone; red triangle points to area of tibial mineralization.



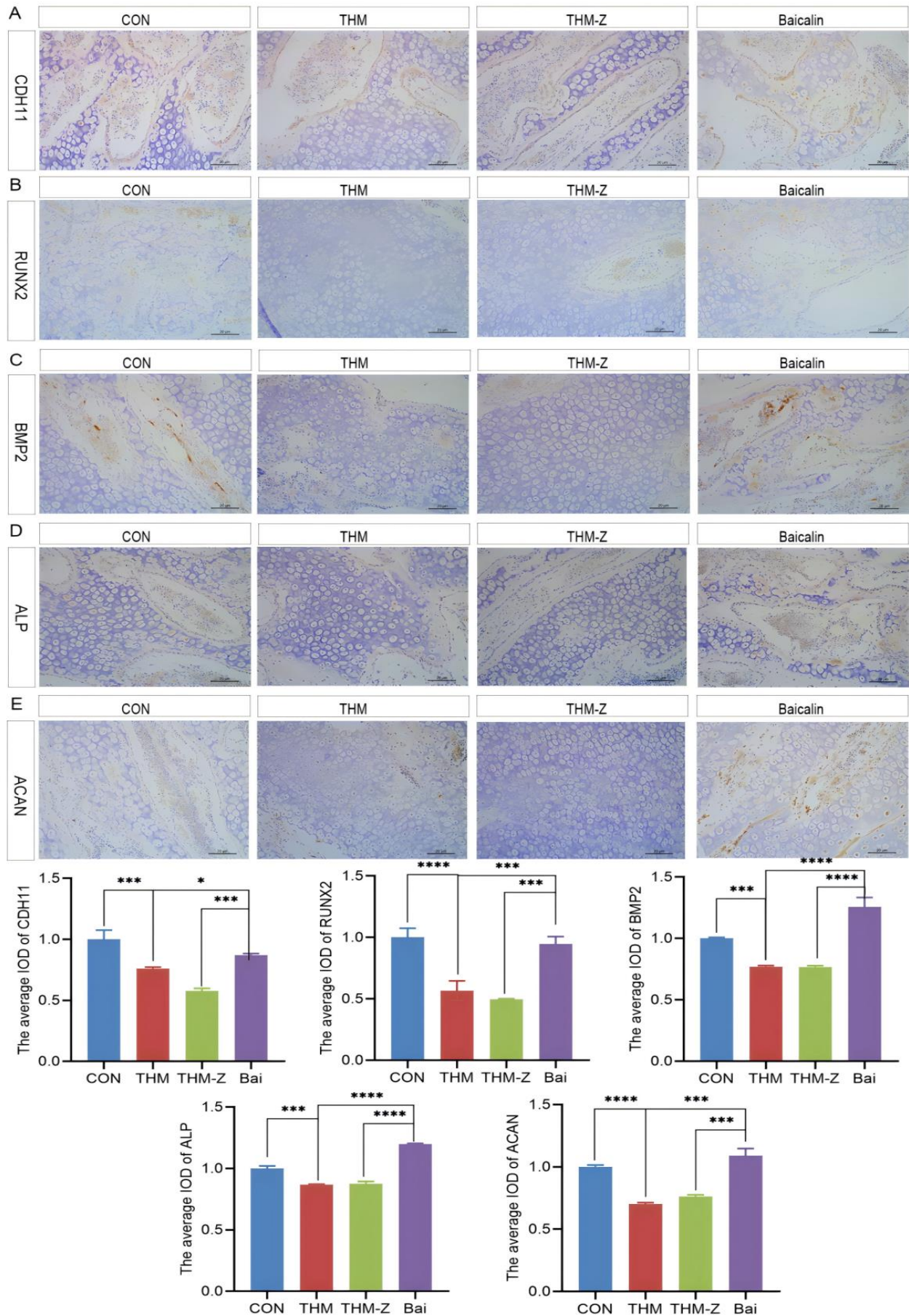


**Fig. 5:** Tibial tissue stained with toluidine blue. A) Histochemical analysis of tibial specimens with toluidine blue staining in control group. B) Histochemical analysis of tibial specimens with toluidine blue staining in TD group. C) Histochemical analysis of tibial specimens with toluidine blue staining in TD self-healing group. D) Histochemical analysis of tibial specimens with toluidine blue staining in baicalin group.



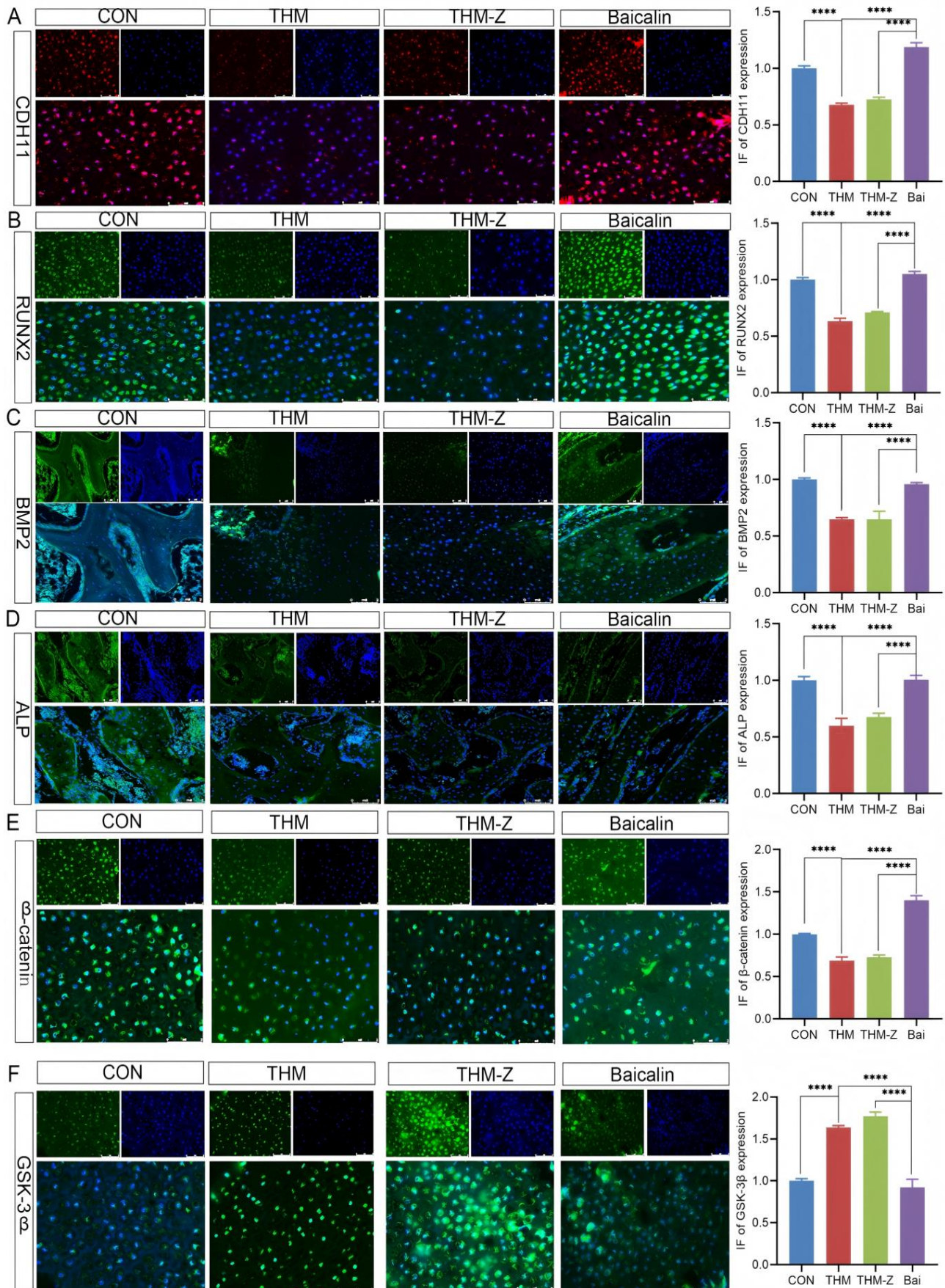
**Fig. 6:** Tibial development related genes and protein expression levels. A) Tibial development-related gene CDH11 expression level. B) Tibial development-related gene β-catenin expression level. C) The expression profiles of the proteins involved in tibial morphogenesis. D) The expression profiles of the proteins involved in the tibial development-related pathway. One-way ANOVA with Tukey's test was used for multiple comparisons. Data are presented as the mean ± SEM. n=3. ns means no significant difference; \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001.





**Fig. 7:** Immunohistochemical map of proteins associated with tibial cartilage tissue development in broiler chickens. A) CDH11 protein immunohistochemistry. B) RUNX2 protein immunohistochemistry. C) BMP2 protein immunohistochemistry. D) ALP protein immunohistochemistry. E) ACAN protein immunohistochemistry. One-way ANOVA with Tukey's test was used for multiple comparisons. Data are presented as the mean  $\pm$  SEM.  $n=3$ . \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ , \*\*\*\* $P<0.0001$ .





**Fig. 8:** Immunofluorescence assay for tibial development-related gene proteins. A) Immunofluorescence assay for tibial development-related gene protein CDH11. B) Immunofluorescence assay for tibial development-related gene protein RUNX2. C) Immunofluorescence assay for tibial development-related gene protein BMP2. D) Immunofluorescence assay for tibial development-related gene protein ALP. E) Immunofluorescence assay for tibial development-related gene protein β-catenin. F) Immunofluorescence assay for tibial development-related gene protein GSK-3β. One-way ANOVA with Tukey's test was used for multiple comparisons. Data are presented as the mean ± SEM. \*\*\*\*P<0.0001.

## DISCUSSION

When broiler chickens suffer from tibial cartilage dysplasia, immature chondrocytes in the growth plate cartilage proliferation area grow excessively, forming cartilage plugs. Previous studies have found that the gene expression of chicken tibial growth plate chondrocytes undergoes significant changes during TD, and the protein composition of cartilage matrix also changes accordingly (Yao *et al.*, 2023). The developmental trajectory of endochondral ossification is governed by a network of signaling molecules, including transcription factors (RUNX2) and growth factors (BMP2, Wnt4), which modulate the canonical  $\beta$ -catenin signaling pathway through GSK-3 $\beta$  regulation. This coordinated interplay orchestrates sequential biological processes such as chondrocyte maturation, osteoblast specification, and proliferative activity during skeletal morphogenesis (Salhotra *et al.*, 2020). RUNX2 is significantly expressed in the hypertrophic zone of chondrocytes, suggesting that RUNX2 is closely related to chondrogenic processes (Wu *et al.*, 2024). BMPs mediate proliferation, differentiation, and invasive processes of cells through canonical Smad signaling and non-canonical MAPK pathways, with their osteogenic activity being harnessed for bone tissue engineering applications (Xing *et al.*, 2025). In this study, TD induced abnormal protein secretion in chondrocytes, including cadherin CDH11, RUNX2, BMP2, etc., concurrently inducing dysregulation of core components within the Wnt/ $\beta$ -catenin signaling cascade,  $\beta$ -catenin, Wnt4a, and GSK-3 $\beta$ . These molecular mediators coordinate and control a comprehensive regulatory network of skeletal cell dynamics, including the differentiation and proliferation of chondrocytes and osteoblasts. CDH11 is a classical type II calreticulin, first identified in mouse osteoblasts, with specific functions in bone development and maintenance. The expression of CDH11 decreased in broiler chickens with TD, indicating that TD inhibits chondrocyte differentiation.  $\beta$ -catenin binds to the carboxyl-terminal fragment (CTF1/2) produced by CDH11 cleavage at the cell membrane, and the cytoplasmic stabilization of  $\beta$ -catenin mediated by CDH11-CTF/ $\beta$ -catenin complexes facilitates its nuclear translocation, thereby initiating transcriptional activation of Wnt signaling pathways (Liu *et al.*, 2023). The canonical Wnt/ $\beta$ -catenin signaling cascade orchestrates critical biological functions, including oncogenic proliferation regulation and cellular differentiation dynamics, and has become an important target for clinical tumor-targeted therapy (Zhang and Wang, 2020).  $\beta$ -catenin is a key mediator of the Wnt/ $\beta$ -catenin signaling pathway, which can regulate the stability of the disruption complex (Yu *et al.*, 2021). The classical Wnt/ $\beta$ -catenin signaling pathway is activated through a multifaceted mechanism mediated by diverse signaling components, including ligand-receptor interactions and intracellular regulatory molecules. One of the reasons for this phenomenon is the phosphorylation and proteasomal degradation of  $\beta$ -catenin (Liu *et al.*, 2023). The canonical Wnt/ $\beta$ -catenin pathway is critically involved in orchestrating embryonic joint morphogenesis and maintaining adult skeletal homeostasis (Yuasa *et al.*, 2008). In addition, the upregulation of Wnt/ $\beta$ -catenin signaling triggers transcriptional activation of MMPs and other proteolytic enzymes, thereby resulting in the breakdown of matrix

proteoglycans (Guo *et al.*, 2025). GSK-3 $\beta$  is involved in various intracellular signaling pathways, suppressing the canonical Wnt/ $\beta$ -catenin signaling pathway through attenuation of  $\beta$ -catenin cytoplasmic accumulation and nuclear translocation (Libro *et al.*, 2016). Lithium chloride reduces the activity of GSK-3 $\beta$  by increasing its phosphorylation and directly activating the Akt pathway (Vallée *et al.*, 2021). According to the experimental results of this article, it was found that gene activity associated with tibial cartilage development and Wnt4 and  $\beta$ -catenin were upregulated after treatment with baicalin, while gene activity associated with GSK-3 $\beta$  was downregulated, indicating that baicalin can act as an activator of the Wnt/ $\beta$ -catenin signal transduction pathway. The pathway can regulate chondrocyte differentiation and mediate the interaction between chondrocytes and osteoblasts in tibial growth plates (Marini *et al.*, 2023). So, when the pathway was activated, genes implicated in chondrogenesis exhibited elevated transcriptional activity, indicating that this signaling pathway promotes the development of tibial cartilage.

Baicalin, as an effective ingredient of the traditional medicinal plant *Scutellaria baicalensis* (Wan *et al.*, 2024), has obvious therapeutic effects on diseases. When broiler chickens develop TD, they usually exhibit mental depression, unwillingness to move or stand, reduced food intake, followed by swelling of leg joints and a split posture (Sun *et al.*, 2023). After treatment with baicalin, broiler chickens showed significant mental recovery, reduced leg joint swelling, and were gradually able to stand. Baicalin enhances osteogenic differentiation of odontoblasts via the canonical Wnt/ $\beta$ -catenin signaling cascade (Kimura *et al.*, 2018). Studies have shown that baicalin has the strongest stimulating effect on ALP activity and can significantly upregulate osteogenic differentiation markers (including COL1a1, osteocalcin, and osteopontin) during osteoblast differentiation, while concurrently promoting mineralization capacity of bone-forming cells (Guo *et al.*, 2011). At the same time, baicalin can activate the Wnt/ $\beta$ -catenin pathway, increase cell viability, enhance osteoblast activity, and improve the expression levels of RUNX2 and osteocalcin in primary osteoblasts (Lin *et al.*, 2020). We found that the clinical symptoms of TD broiler chickens gradually improved after feeding baicalin. Moreover, the upregulation of gene expression related to cartilage development indicates that baicalin can promote cartilage development and reverse TD induced by thiram in broiler chickens. This study thoroughly evaluated the therapeutic effect of baicalin on TD. It elucidated the mechanism by which baicalin affects cartilage development by regulating the Wnt/ $\beta$ -catenin pathway, establishing a reference and basis for designing pharmaceutical interventions targeting broiler poultry.

**Conclusions:** This study systematically demonstrates the therapeutic potential of baicalin against tibial dyschondroplasia (TD) in broilers. Clinical observations revealed that baicalin administration effectively reversed TD-induced pathological manifestations, including leg joint swelling and movement disorders. Investigations identified the Wnt/ $\beta$ -catenin pathway as a core molecular target, where baicalin significantly upregulated  $\beta$ -catenin nuclear translocation and enhanced the expression of cartilage development regulators. These actions establish baicalin as a novel phytotherapeutic candidate that not only



rescues TD but also reconstructs the developmental microenvironment through pathway-specific modulation. The findings provide both theoretical foundations for understanding TD pathogenesis and practical solutions for developing natural product-based alternatives.

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Acknowledgement:** This work was financially supported by the XiZang Natural Science Foundation (XZ202501ZR0025), the National Natural Science Foundation of China (No. 32273073; 32002350).

**Author contributions:** K.L. and A.L.: Conceptualization, Investigation, Methodology, Data curation, Project administration, Visualization, Writing-original draft. Y.L.: Investigation, Data curation. H.Z., K.M. and Y.S.: Writing-review and editing.

**Ethics approval:** The protocols were approved by the South China Agricultural University Committee on Investigations Involving Animal Subjects. All experiments were approved by the Animal Care Committee of South China Agricultural University (permit number: 32002350)

## REFERENCES

- Feng H, Zhang J, Wang X *et al.*, 2024. Baicalin protects broilers against avian coronavirus infection via regulating respiratory tract microbiota and amino acid metabolism. *Int J Mol Sci* 25(4): 2109.
- Guo AJ, Choi RC, Cheung AW *et al.*, 2011. Baicalin, a flavone, induces the differentiation of cultured osteoblasts: an action via the Wnt/ $\beta$ -catenin signaling pathway. *J Biol Chem* 286(32):27882-27893.
- Guo S, Sidhu R, Ramar V *et al.*, 2025. RNA sequencing identifies novel signaling pathways and potential drug target genes induced by FOSL1 in glioma progression and stemness. *Biologics* 5(19):157-176.
- Iqbal M, Waqas M, Mo Q *et al.*, 2023. Baicalin inhibits apoptosis and enhances chondrocyte proliferation in thiram-induced tibial dyschondroplasia in chickens by regulating Bcl-2/Caspase-9 and Sox-9/Collagen-II expressions. *Ecotoxicol Environ Saf* 268(1):115689.
- Janczi T, Böhm B, Fehrl Y *et al.*, 2025. Mechanical forces trigger invasive behavior in synovial fibroblasts through N-cadherin/ADAM15-dependent modulation of lncRNA H19. *Sci Rep* 15(1):9814.
- Kianfar R, Kanani R, Janmohammadi H *et al.*, 2025. Implications of high-dose vitamin D3 with and without vitamin C on bone mineralization and blood biochemical factors in broiler breeder hens and their offspring. *Peer J* 4(13):e18983.
- Kimura A, Kunimatsu R, Yoshimi Y *et al.*, 2018. Baicalin promotes osteogenic differentiation of human cementoblast lineage cells via the wnt/ $\beta$  catenin signaling pathway. *Curr Pharm Des* 24(33):3980-3987.
- Li J, Cao H, Zhou X *et al.*, 2023. Advances in the study of traditional Chinese medicine affecting bone metabolism through modulation of oxidative stress. *Front Pharmacol* 14(1):1235854.
- Li J and Hao J, 2019. Treatment of neurodegenerative diseases with bioactive components of tripterygium wilfordii. *Am J Chin Med* 47(4):769-785.
- Libro R, Bramanti P and Mazzon E, 2016. The role of the Wnt canonical signaling in neurodegenerative diseases. *Life Sci* 158(1):78-88.
- Lin P, Guo XX, Wang YL *et al.*, 2020. Inhibitory effect of baicalin on orthodontically induced inflammatory root resorption in rats. *J Int Med Res* 48(9):1220754622.
- Liu Y, Lei P, Samuel RZ *et al.*, 2023. Cadherin-11 increases tumor cell proliferation and metastatic potential via Wnt pathway activation. *Mol Oncol* 17(10):2056-2073.
- Manohar S, Camacho-Magallanes A, Echeverria C Jr *et al.*, 2020. Cadherin-11 is required for neural crest specification and survival. *Front Physiol* 11(1):563372.
- Marini F, Giusti F, Palmini G *et al.*, 2023. Role of Wnt signaling and sclerostin in bone and as therapeutic targets in skeletal disorders. *Osteoporos Int* 34(2):213-238.
- Mehmood K, Zhang H, Yao W *et al.*, 2019. Protective effect of Astragaloside IV to inhibit thiram-induced tibial dyschondroplasia. *Environ Sci Pollut Res Int* 26(16):16210-16219.
- Molagoda IMN, Kang CH, Lee MH *et al.*, 2021. Fisetin promotes osteoblast differentiation and osteogenesis through GSK-3 $\beta$  phosphorylation at Ser9 and consequent  $\beta$ -catenin activation, inhibiting osteoporosis. *Biochem Pharmacol* 192(1):14676.
- Osiak-Wicha C, Kras K, Tomaszewska E *et al.*, 2025. Evaluation of PTSD-Induced alterations in bone biomechanics and the protective potential of CE-123 in a wistar rat model. *J Clin Med* 14(7):2427.
- Passanha FR, Divinagracia ML and LaPointe VLS, 2022. Cadherin-11 regulates cell proliferation via the PDGFR $\beta$ -ERK1/2 signaling pathway in human mesenchymal stem cells. *Stem Cells* 40(2):165-174.
- Passanha FR, Geuens T and LaPointe VLS, 2022. Cadherin-11 influences differentiation in human mesenchymal stem cells by regulating the extracellular matrix via the TGF $\beta$ 1 pathway. *Stem Cells* 40(7):669-677.
- Pham NT, Le HG, Peng BR *et al.*, 2025. Computational analysis of zoanthamine alkaloids from *Zoanthus* sp. as potential DKK1 and GSK-3 $\beta$  inhibitors for osteoporosis therapy via Wnt signaling. *Sci Rep* 15(1):14297.
- Sahin E, Ipcak HH, Orhan C *et al.*, 2024. Impact of the arginine silicate inositol complex on bone metabolism in broiler chickens with tibial dyschondroplasia caused by manganese deficiency. *Br Poult Sci* 65(4):455-464.
- Salhotra A, Shah HN, Levi B *et al.*, 2020. Mechanisms of bone development and repair. *Nat Rev Mol Cell Biol* 21(11):696-711.
- Shen J, Sun Y, Liu X *et al.*, 2021. EGFL6 regulates angiogenesis and osteogenesis in distraction osteogenesis via Wnt/ $\beta$ -catenin signaling. *Stem Cell Res Ther* 12(1): 415.
- Shi K, Wu Y, Jiang X *et al.*, 2024. Transcriptome analysis reveals the pathogenesis of spontaneous tibial dyschondroplasia in broilers. *Front Genet* 29(15):1434532.
- Sun Q, Wu S, Liu K *et al.*, 2023. miR-181b-1-3p affects the proliferation and differentiation of chondrocytes in TD broilers through the WIF1/Wnt/ $\beta$ -catenin pathway. *Pestic Biochem Physiol* 197(1):105649.
- Vallée A, Vallée JN and Lecarpentier Y, 2021. Parkinson's disease: potential actions of lithium by targeting the wnt/ $\beta$ -Catenin pathway, oxidative stress, inflammation and glutamatergic pathway. *Cells* 10(2):230.
- Wan S, Wang L, Hao Z *et al.*, 2024. Baicalin ameliorates the gut barrier function and intestinal microbiota of broiler chickens. *Acta Biochim Biophys Sin (Shanghai)* 56(4):634-644.
- Wang Q, Shi D, Geng Y *et al.*, 2020. Baicalin augments the differentiation of osteoblasts via enhancement of microRNA-217. *Mol Cell Biochem* 463(1):91-100.
- Wu S, Liu K, Huang X *et al.*, 2024. Molecular mechanism of miR-203a targeting Runx2 to regulate thiram induced-chondrocyte development. *Pestic Biochem Physiol* 200(1):105817.
- Wu X, Liu Y, Li Y *et al.*, 2024. Molecular mechanism of thiram-induced abnormal chondrocyte proliferation via lncRNA MSTRG.74.1-BNIP3 axis. *Pestic Biochem Physiol* 201(1):105847.
- Xiao Y, Guan T, Yang X *et al.*, 2023. Baicalin facilitates remyelination and suppresses neuroinflammation in rats with chronic cerebral hypoperfusion by activating Wnt/ $\beta$ -catenin and inhibiting NF- $\kappa$ B signaling. *Behav Brain Res* 442(1):114301.
- Xing F, Liu Y, Tian F *et al.*, 2025. BMP2 expression in oral squamous cell carcinoma and its effects on SCC9 cell biological behavior. *Sci Rep* 15(1):11584.
- Yao W, Kulyar MF, Ding Y *et al.*, 2023. The Effect of miR-140-5p with HDAC4 towards Growth and Differentiation Signaling of Chondrocytes in Thiram-Induced Tibial Dyschondroplasia. *Int J Mol Sci* 24(13):10975.
- Yu F, Yu C, Li F *et al.*, 2021. Wnt/ $\beta$ -catenin signaling in cancers and targeted therapies. *Signal Transduct Target Ther* 6(1):307.
- Yuasa T, Otani T, Koike T *et al.*, 2008. Wnt/ $\beta$ -catenin signaling stimulates matrix catabolic genes and activity in articular chondrocytes: its possible role in joint degeneration. *Lab Invest* 88(3):264-274.
- Zhang Y and Wang X, 2020. Targeting the Wnt/ $\beta$ -catenin signaling pathway in cancer. *J Hematol Oncol* 13(1):165.
- Zhao Y, Wang HL, Li TT *et al.*, 2020. Baicalin ameliorates dexamethasone-induced osteoporosis by regulation of the RANK/RANKL/OPG signaling pathway. *Drug Des Devel Ther* 14(1):195-206.