



Research article

Comparison of growth promotion effects: Fermented non-medicinal parts of *Scutellaria baicalensis* vs. non-critical rare earth cerium oxide in yellow-feathered broilers

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Abstract

This study investigated the effects of fermented *Scutellaria baicalensis* stem and leaves and Cerium Oxide (CeO₂) supplementation on the growth performance, slaughter performance, and immune function of yellow-feather broilers. The results demonstrated that the average daily feed intake (ADFI) was significantly higher ($P < 0.05$) in the treatment groups compared to the antibiotic-positive group. The group treated with 0.8% fermented *Scutellaria baicalensis* stems and leaves exhibited the high average daily weight gain and feed conversion ratio, surpassing the control group by 3.2% and 2.3%, respectively. Slaughter performance showed that the 0.8% *Scutellaria baicalensis* treatment group had greater chest depth, keel length, pelvic width, and tibia length than other groups. In contrast, the 500 mg CeO₂ treatment group exhibited the longest tibia length. Additionally, the slaughter rates of all treatment groups were significantly higher ($P < 0.0001$) than those of the control and antibiotic-positive groups. Immune function analysis revealed no significant differences ($P > 0.05$) in thymus and bursa indices; however, the thymus index was higher in groups with 0.8% *Scutellaria baicalensis* stem and leaves and CeO₂ groups. Serum immunoglobulin analysis indicated that IgM and IgG levels did not significantly differ among groups, but CeO₂ supplementation increased IgA levels, particularly in the 50 mg and 100 mg groups. These results illustrated that adding 0.8% fermented *Scutellaria baicalensis* stems and leaves to poultry feed effectively enhances improve the growth performance and carcass traits of yellow-feather broilers, and is a potential substitute for antibiotics in poultry production, compared to CeO₂ and Lincomycin hydrochloride.

Keywords: Cerium oxide, Fermented *Scutellaria baicalensis* stem and leaf, Growth performance, Immune function.

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INTRODUCTION

Subtherapeutic dose antibiotics are highly effective in promoting the growth of livestock and poultry, and their usage in animal husbandry is substantial, making it a high-risk area for antibiotic overuse. The overuse of antibiotics in animal husbandry is a significant contributor to the development of antimicrobial resistance (AMR), posing a major threat to human public health. It was estimated that the livestock industry uses approximately 76,060 tons of antimicrobial active ingredients annually, of which 40,697 tons (53.5%) were for cattle, 4,243 tons (5.6%) for chickens, and 31,120 tons (40.9%) for pigs (Ardakani et al., 2024). The current death toll caused by bacterial resistance has become the second leading cause of death globally (GBD 2019 Antimicrobial Resistance Collaborators, 2022). The World Health Organization (WHO) reported that bacterial antimicrobial resistance (AMR) is estimated to directly cause 1.27 million deaths globally each year and contribute to an additional 4.95 million deaths annually (WHO, 2024).

Since 2006, the European Union has banned antibiotics in animal feed, followed by the United States in 2014 and China in 2020. While these bans aimed to reduce antibiotic overuse, they also posed challenges for the livestock industry, which was essential for livelihoods but operated with low-profit margins and high risks. The removal of antibiotic growth promoters threatened the industry's profitability, highlighting the need for sustainable alternatives to balance public health and economic stability. In the meantime, research on various growth promoting agents such as traditional herb medicine and rare earths has been reported (Abdelnour et al., 2019; Wang et al., 2019). Livestock growth promoters typically need to ensure effectiveness, cost, safety, and other key aspects. For growth promoters derived from traditional Chinese medicine, the waste portion can be a valuable option. *Scutellaria baicalensis* is a traditional Chinese medicine that is clinically used primarily as the dried root, while the aerial parts (stems and leaves), regarded as non-medicinal. It can effectively promote animal growth and improve the production performance of dairy cows (Yin et al., 2021). The primary bioactive compounds in the plant are baicalein and baicalin. The roots of *S. baicalensis* contain higher concentrations of these active compounds compared to the stems and leaves. Additionally, it has been reported that fermented *S. baicalensis* extract (FSE) exhibited greater potential anti-acne effects than non-fermented *S. baicalensis* extract (NSE) (Zhu et al., 2020). Therefore, fermenting the aerial parts of *S. baicalensis* may enhance their growth-promoting effects. On the other hand, CeO₂, a non-critical rare earth elements, possesses unique physical and chemical properties that offer significant potential for applications in agriculture. It was reported, in livestock farming, the addition of an appropriate amount of CeO₂ can help regulate animal metabolism, thereby influencing nutrient absorption and utilization (Bölükbaşı et al., 2016; Nockels, 1996). This may ultimately enhance the growth performance of animals.

Animal growth promoters are often selected based on lower cost materials due to profit considerations. Both non-medicinal traditional Chinese medicine and non-critical rare earth elements are viable options. However, previous evaluations have only compared them separately, and no study has yet compared them within the same experiment. To conduct a parallel comparison of the growth promoting effects of non-medicinal traditional Chinese medicine and non-critical rare earth elements, the following experimental design was carried out. The fermented *S. baicalensis* stems and leaves and CeO₂ were used as feed additives to investigate their effects on body weight gain in yellow-feathered broiler chickens. The aim was to preliminarily assess the differences in their efficacy and provide data to support related research.

MATERIALS AND METHODS

Experimental material

Seven-week-old yellow-feathered broiler chickens were sourced from a commercial hatchery in Kunming, Yunnan Province, China. *S. baicalensis* stems and leaves were obtained from Yaoan, Chuxiong City, Yunnan Province. CeO₂ (The purity is 99%, containing 1% zinc oxide) was provided by Inner Mongolia Rare Angel Enterprise Management Co., Ltd. The *Bacillus subtilis* (No. BNCC188080) was preserved in Yunnan Joint International R&D Center of Veterinary Public Health.

Fermentation of *S. baicalensis* stems and leaves: *B. subtilis* was streaked and cultured in Tryptic Carcass Soya Agar (TSA, Hai Bo Biotechnology Co., Qingdao, China), incubated in a thermostat for 12-18 h, and individual *B. subtilis* colonies were picked and placed in test tubes containing Tryptic Carcass Soya Broth (TSB Hai Bo Biotechnology Co., Qingdao, China) nutrient solution, and incubated on a shaker for 12-18 h. The *S. baicalensis* stems and leaves were loaded into a fermenter and sterilized in an autoclave (121 °C for 15 min). Then the sterilized *S. baicalensis* stems and leaves were inoculated with activated *B. subtilis*, and fermented at 25 ± 2 °C for 3 days, then dried (at 70°C for 24 h) and crushed.

Feeding management

The chicken house and equipments were strictly cleaned and sterilized before entering the chickens. Square chicken cages were used in the experiment. The temperature was maintained at about 33-35°C during the pre-feeding period, and then decreased by 2°C per week until it was reduced to room temperature. Relative humidity was controlled at 65-70%. Natural ventilation, free feeding and drinking, and regular cleaning of the pen were used during the test period. The growth condition and mental state of the chickens were observed every day.

Experiment design and sampling

The experiment was designed as a one-way trial, and 90 healthy, well-bodied, white-feathered broilers of 7 weeks of age with close body weights were selected and randomly divided into 9 groups of 10 birds each. The *Scutellaria baicalensis* stems and leaves group and the CeO₂ group were each divided into three dosage levels: high, medium, and low. Additionally, there was a control group and two antimicrobial groups, L-LH and H-LH (2.55×10⁻¹ g and 3.33×10⁻⁴ g Lincomycin hydrochloride). The experiment was conducted for a total of 18 d, including a pre-feeding period of 4 d and an experimental period of 14 d. The control group was fed a basal diet (CP FEED NO. 523, Charoen Pokphand Group Co., Ltd.). The fermented *S. baicalensis* stem and leaf group was fed a basal diet supplemented with 0.4%, 0.8%, and 1.2%, abbreviated as 0.4%Sb, 0.8%Sb, and 1.2%Sb, respectively. The CeO₂ group was fed basal feed supplemented with CeO₂, at concentrations of 50 mg, 100 mg, and 500 mg, abbreviated as L-CeO₂, M-CeO₂, and H-CeO₂, respectively.

The feed consumption and the weight were recorded every morning on an empty stomach. On day 14 of the experiment, 10 mL of blood was collected from each tested chicken *via* venipuncture under the wing vein. The blood was divided into two tubes: one with EDTA anticoagulant (shaken gently to mix) and the other without anticoagulant, left at room temperature to separate serum. The latter was then centrifuged at 3000 g/min for 10 min using a centrifuge (Bellman Coulter, USA). Then the serum was stored at 4°C for the ELISA test. The chickens were euthanized by bleeding from the carotid artery, then dissected. The bursa of Fabricius and thymus were weighed to assess immune indicators. The experiment ethics was approved by the Bioethics Review Committee of Yunnan Agricultural University (Reference No. 202103003).

Growth performance

The growth parameters evaluated were as follows: Average daily gain (ADG) = (average weight at the end of the experiment - average weight at the beginning of the experiment) / number of days in the experiment; Average Daily Feed Intake (ADFI) = Total Feed Intake / Experimental Period; Feed Conversion Ratio (FCR) = Feed Intake (F) / Weight Gain (G).

Slaughter performance

The following parameters of the experimental chickens were measured: body slant length, breast width, breast depth, keel length, pelvic width, shank length, shank circumference, live weight, carcass weight, half-cleaned weight, and full-cleaned weight. The Half-cleaned rate = (half-cleaned weight)/live weight×100%; Full-cleaned rate = (full-cleaned weight)/live weight×100%; Slaughter rate = carcass weight/livestock live weight × 100%.

Immune function

The immune organ index (IOI) evaluated were as follows: Bursa index = (bursa weight/slaughter weight); thymus index = (thymus weight/slaughter weight).

The blood was collected into an EDTA anticoagulant tube and then analyzed using a fully automatic animal blood cell analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.) to determine the levels of white blood cells (WBC), red blood cells (RBC), and lymphocytes. The serum stored at 4 °C was used to determine IgA, IgM and IgG by the ELISA kit (Enzyme Immunoassay Industrial Co., Ltd., Jiangsu, China).

Statistical analysis

The experiment was statistically analyzed by one-way ANOVA using SPSS 19.0 software. Multiple comparisons were performed using the Duncan method. The results were compared by the mean difference±standard deviation. The P value less than 0.05 was considered as significant difference.

RESULTS

Effects of fermented *S. baicalensis* stem leaves and Cerium Oxide on the growth performance of yellow-feather broilers

The results were shown in Table 1. The average daily feed intake (ADFI) was significantly higher ($P < 0.0001$) in both the experimental and control group compared to the antibiotic-positive group, while ensuring that there were no significant differences in the initial body weights among the groups. The groups treated with 0.8% Sb and H-CeO₂ had higher average daily feed intake compared to the other groups. The average daily feed intake of the control group was 2.66% and 3.22% higher than that of the 0.8% Sb group and the M-CeO₂ group, respectively. The average daily feed intake of the group treated with 0.8% Sb was also higher than that of the CeO₂ and antimicrobial groups. The average daily weight gain and feed conversion ratio of the group treated with 0.8% Sb extract were both higher than those of other groups, with increases of 3.2% and 2.3% compared to the control group, respectively.

Table 1 Growth performance of fermented stem leaves of *Scutellaria baicalensis* and Cerium Oxide on yellow feather broilers

Item	control	0.4% Sb	0.8% Sb	1.2% Sb	L-CeO ₂	M-CeO ₂	H-CeO ₂	L- LH	H-LH	P-value
IW (kg)	0.55 ± 0.072	0.52 ± 0.04	0.53 ± 0.05	0.52 ± 0.05	0.53 ± 0.07	0.54 ± 0.05	0.52 ± 0.03	0.53 ± 0.05	0.52 ± 0.08	/
FW (kg)	1.07 ± 0.16	0.93 ± 0.14	1.08 ± 0.13	0.96 ± 0.14	0.96 ± 0.21	1.01 ± 0.16	1.04 ± 0.07	0.96 ± 0.15	0.91 ± 0.16	0.67
ADFI (g)	98.06 ± 2.3 ^c	95.52 ± 0.54 ^d	101.8 ± 0.41 ^a	98.5 ± 0.32 ^{bc}	99.55 ± 0.53 ^b	95.0 ± 0.33 ^{de}	101.6 ± 0.61 ^a	93.64 ± 0.34 ^e	91.74 ± 0.66 ^f	P<0.0001
ADG (g)	37.98 ± 8.5	29.60 ± 8.91	39.20 ± 7.14	31.29 ± 9.30	30.75 ± 11.6	34.0 ± 9.7	37.49 ± 4.38	30.8 ± 10.1	27.3 ± 8.68	0.112
FCR	3.52 ± 1.19	2.68 ± 0.54	3.6 ± 1.83	3.8 ± 2.0	3.0 ± 0.80	2.78 ± 0.33	2.74 ± 0.83	3.3 ± 0.92	3.71 ± 1.37	0.3733

Note: Values were expressed as mean ± SD. Data with different letters were significantly different ($P < 0.05$) among; LH shorted as Lincomycin hydrochloride; IW (kg) shorted as treatments. Initial weight (kg); FW (kg) shorted as Final weight (kg); ADFI (g) shorted as average daily feed intake (g); ADG (g) shorted as average daily gain (g); FCR is mean feed conversion ratio.

Effect on slaughter performance

The results are shown in [Figure 1](#). There were no significant differences ($P > 0.05$) in chest depth, keel length, pelvic width, tibia length, and tibia circumference among the groups. Notably, the 0.8% Sb group exhibited higher values in chest depth, keel length, pelvic width, and tibia length compared to all other groups. The body oblique length and chest width of fermented *S. baicalensis* stems and leaves supplemented with 0.8% differed significantly from those of the positive group supplemented with 3.33×10^{-4} g of antibiotic ($P < 0.05$). The body oblique length, chest length, and pelvic width of the treatment group supplemented with 100 mg of CeO₂ were higher than those of the control group. Additionally, the tibia length of the treatment group supplemented with 500 mg of CeO₂ was higher than that of all other experimental groups.

According to [Figure 2](#), there was little difference between the partially eviscerated poultry rate and fully eviscerated poultry rates across different treatment groups, with all semi-clean rates ranging from 70% to 80%, and full-clean rates ranging from 60% to 70%. Notably, significant differences were observed in slaughter rates among the treatment groups. The slaughter rates of the six treatment groups were significantly higher ($P < 0.0001$) than those of the control and positive antibiotic treatment groups.

Effects on immune function

The results are shown in [Figure 3](#). There was no significant difference ($P > 0.05$) in the immune organ indices: thymus index and bursa index of each group. The thymus indices of the 0.8% fermented *S. baicalensis* stems and leaves treatment (0.8% Sb) group and the treatment groups with different concentrations of CeO₂ were higher than those of the control group, while the thymus indices of the other treatment groups were lower than those of the control group. The thymus index was higher in the treatment group with 50 mg and 500 mg of CeO₂ than in the control group.

The analysis of serum immunoglobulins revealed no significant differences in IgM and IgG levels among the groups ($P > 0.05$). Overall, IgM levels tended to be lower in the experimental groups compared to the control group. Notably, within the experimental treatments, the groups supplemented with CeO₂ showed relatively higher IgM levels than those receiving *S. baicalensis*.

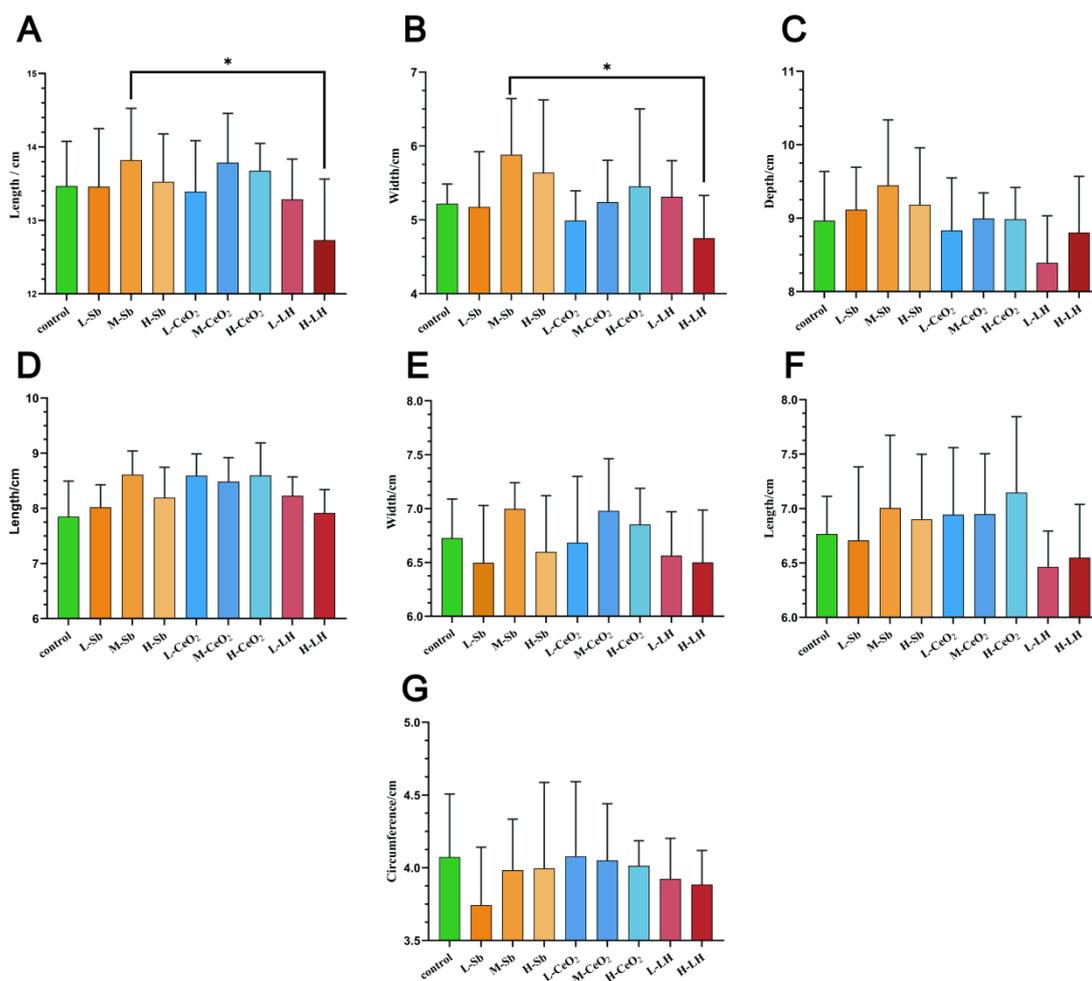


Figure 1 Effects of fermented *Scutellaria baicalensis* stems and leaves and CeO₂ on the growth and body size of yellow-feathered broilers. The results of body oblique length measurements for each experimental group are shown in Panel A. The results of chest width measurements are displayed in Panel B. Chest depth measurements are illustrated in Panel C, while keel length measurements are presented in Panel D. Pelvic width measurements are shown in Panel E, tibia length measurements are displayed in Panel F, and tibia circumference measurements are presented in Panel G. Data were expressed as mean \pm SD ($n = 30$), * indicated significant difference in data ($P < 0.05$).

Specifically, compared to the control group, supplementation with 50 mg of CeO₂ (L-CeO₂) increased IgA levels by 37%, while supplementation with 100 mg of CeO₂ (M-CeO₂) resulted in a 22.9% increase in IgA levels. Notably, the IgA level in the 50 mg CeO₂ (L-CeO₂) treatment group was significantly different from that in the positive control group (L-LH) and the 1.2% fermented *S. baicalensis* stem-leaf group (1.2% Sb) ($P < 0.0001$). Additionally, it was significantly different from the 1.2% fermented *S. baicalensis* stem-leaf (1.2% Sb) group ($P < 0.05$) and significantly higher than that in the 500 mg CeO₂ (H-CeO₂) treatment group ($P < 0.01$). Furthermore, the IgA level in the 100 mg CeO₂ (M-CeO₂) treatment group was significantly different from those in both the positive control group (L-LH) and the 1.2% fermented *S. baicalensis* stem-leaf (1.2% Sb) group ($P < 0.01$).

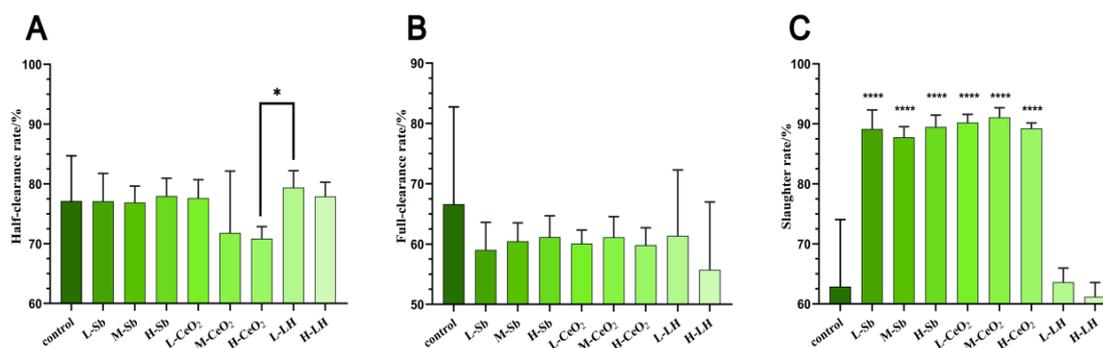


Figure 2 Effects of fermented *Scutellaria baicalensis* stems and leaves and CeO₂ on the growth performance of yellow-feathered broiler chickens. The half-clearance rate of each experimental group was shown in Panel A; the full-clearance rate of each experimental group was shown in Panel B; and the slaughtering rate of each experimental group was shown in Panel C. The values were expressed as mean \pm SD (n = 30). Significant differences in data (P < 0.05) were denoted by asterisks (*), P < 0.05 (*); P < 0.01 (**); P < 0.001 (**); P < 0.0001 (****).

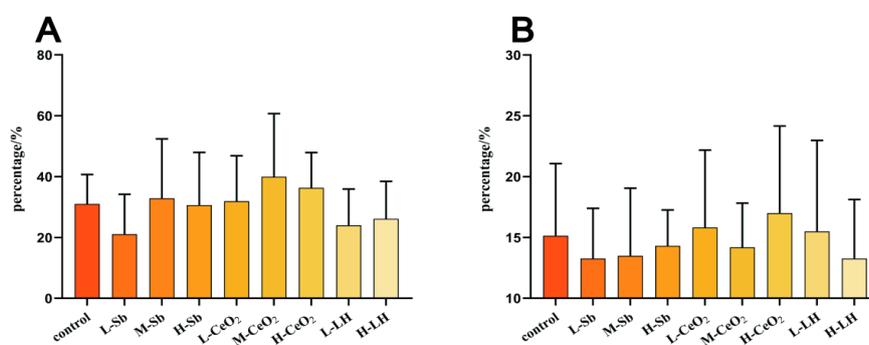


Figure 3 Effects of fermented *Scutellaria baicalensis* stems and leaves and CeO₂ on immune organs of yellow-feathered broilers. The thymus index results for each experimental group were presented in Panel A, and the bursa index results were presented in Panel B. The values were expressed as mean \pm SD (n = 30). An asterisk (*) was used to denote that the data were significantly different (P < 0.05).

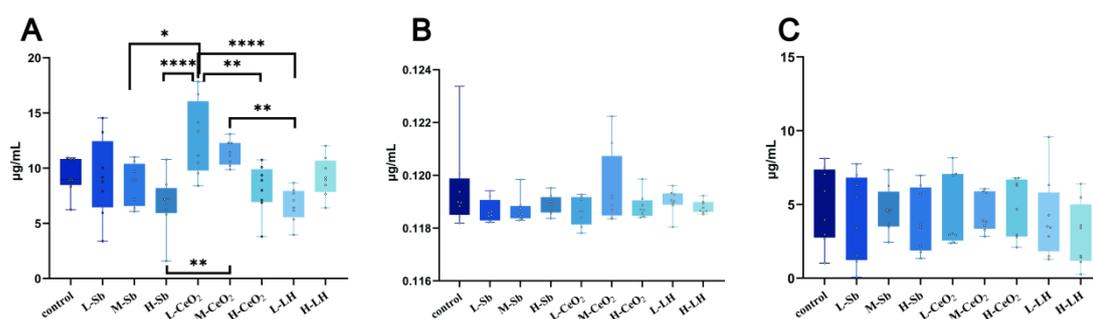


Figure 4 Effects of fermented *Scutellaria baicalensis* stems and leaves and CeO₂ on the growth performance of yellow-feathered broiler chickens. The IgA of each experimental group was shown in Panel A; the IgG of each experimental group was shown in Panel B; and the IgM of each experimental group was shown in Panel C. The values were expressed as mean \pm SD (n = 30). Significant differences in data (P < 0.05) were denoted by asterisks (*), P < 0.05 (*); P < 0.01 (**); P < 0.001 (**); P < 0.0001 (****).

DISCUSSION

This experiment comparatively explored the potential of using the non-medicinal part of fermented *S. baicalensis* and non-critical rare earth element CeO₂ as feed additives for broilers, with the exploration of potentially replacing antibiotics and promoting growth performance. The results of the slaughter indicators, particularly the higher slaughter rates observed in the experimental treatment groups, suggested that the use of fermented *S. baicalensis* stems and leaves, combined with CeO₂ supplementation, may enhance the overall yield and processing efficiency in broilers. Although there were no significant differences in body measurements (e.g. body length and chest width) among the groups, it is worth noting that the shin length of the experimental group supplemented with CeO₂ was higher than that of the other experimental groups, which is similar to the results of previous studies (Sabir and Abbas, 2023). Except for the shin circumference, the treatment group with the addition of 0.8% fermented *S. baicalensis* stem leaves was higher than the other groups, which was consistent with Yin's research results (Yin et al., 2021). There were no significant differences in structural development, indicating that the intervention primarily affected feed intake and growth performance. Nevertheless, it should be noted that most studies did not specifically address the impact on structural development. There is a significant reason for the poor palatability (Pujianti, 2013). This finding highlighted the potential for these additives to positively influence physiological factors that may indirectly enhance meat yield and processing traits (Liu et al., 2023; Zhou et al., 2023). Notably, the significant differences in slaughter rates suggested that these treatments could improve overall meat quality or processing efficiency, which is a critical consideration for the poultry industry (Petracci et al., 2010). The observed improvement in slaughter rates may be attributed to the enhanced overall health and vitality of the broilers resulting from the feed additives, leading to better handling and reduced stress during processing.

The effects of fermented *S. baicalensis* stems and leaves and CeO₂ on the immune organs and immunoglobulins of broilers were evaluated under consistent experimental conditions using multiple parallel groups. This design ensured data comparability and provided greater scientific rigor compared to cross-study comparisons (Nair, 2019). The results indicated that the addition of fermented *S. baicalensis* and CeO₂ at different concentrations had no significant effect on the thymus and bursa indices, consistent with the findings of Fascina's work (Fascina et al., 2017). However, the thymus index was higher in the 0.8% *S. baicalensis* group and the 50 mg and 500 mg CeO₂ groups compared to the control, indicating potential immunomodulatory effects at these specific doses. The flavonoid compounds in *S. baicalensis* (e.g., baicalin) and the antioxidant properties of CeO₂ may have contributed to the promotion of T-cell development and enhanced immune function (Yang, 2012). Conversely, some treatment groups exhibited a lower thymus index than the control group, suggesting a dose-dependent effect, where excessive or insufficient supplementation may impose a metabolic burden or disrupt homeostasis.

The experimental results demonstrated that supplementation with 50 mg and 100 mg CeO₂ significantly elevated serum IgA levels (by 37% and 22.9%, respectively), with the 50 mg dose exhibiting superior efficacy. This phenomenon may be attributed to the antioxidant and immunomodulatory properties of CeO₂ nanoparticles (Allu et al., 2023). Previous studies have confirmed that low-dose CeO₂ nanoparticles alleviate oxidative stress by scavenging reactive oxygen species (ROS), demonstrating the potential for regulating cellular functions (Ribera et al., 2019). However, the diminished effect observed in the 500 mg group suggests potential toxicity or immunosuppression at higher doses, consistent with the "biphasic dose-response" characteristic of nanomaterials (Bailey et al., 2020). The fermented *S. baicalensis* stem-leaf group (1.2%) exhibited significantly lower IgA and IgM levels compared to the CeO₂ groups. This discrepancy may stem from

altered bioavailability of active constituents (e.g., baicalin) during fermentation (Ji et al. 2024). However, the significant increase in IgA levels indicates its potential to enhance mucosal immunity, which plays a key role in defending against infection (El Ansari et al., 2025).

In conclusion, the effects of fermented *S. baicalensis* stems and leaves and CeO₂ on the immune system of broilers demonstrated a dose-dependent pattern, with certain concentrations enhancing immune function. Nevertheless, the overall change was statistically partially significant. Further studies incorporating histological analysis and immune response assessments are warranted to elucidate the underlying mechanisms and determine the optimal supplementation levels. In conclusion, the supplementation of fermented *S. baicalensis* stems and leaves, particularly at 0.8%, as well as CeO₂, demonstrated potential benefits in improving the growth performance and slaughter indicators of yellow-feathered broilers. The increase in feed intake and the potential for enhanced immune responses point toward their utility in improving poultry health and productivity. However, further research is required to confirm the mechanisms behind these effects and to explore optimal dosages and combinations of these additives. Long-term studies would also be beneficial to assess their impacts on poultry health and meat quality over extended production periods.

CONCLUSION

The study comparatively explored the potential of dietary supplementation with fermented *S. baicalensis* stems and leaves and CeO₂ to replace antibiotics and promote growth performance, which exhibited significant improvements in feed intake and slaughter performance, as well as specific immunological effects. Supplementing broiler diets with 0.8% fermented *S. baicalensis* improved growth and slaughter performance, showing more potential as a dietary additive for poultry production than CeO₂ or lincomycin hydrochloride.

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Liu Hongmei: Conceptualization (Lead); Formal analysis (Equal); Writing – review & editing (Supporting).

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Zuo Weidong: Formal analysis (Supporting); Project administration (Supporting).

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Yu Shiwei: Funding acquisition (Supporting); Investigation (Supporting).

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AUTHOR DISCLOSURE STATEMENTS

The authors declare no conflict of interest.

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