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«Research Note»

Supplementation of 5-Aminolevulinic Acid Suppressed Body Weight Loss and Reduced Disease Severity During *Eimeria tenella* Infection in Broiler Chickens

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This study aimed to evaluate the effects of 5-aminolevulinic acid (5-ALA) supplementation in broiler chickens infected with *Eimeria tenella*. To assess these effects, chickens supplemented with 20 ppm 5-ALA (5-ALA group) were compared with non-supplemented controls (control group). Sporulated *E. tenella* oocysts (2.0×10^3 oocysts per animal) were administered orally to 2-week-old broiler chickens. Body weight was measured weekly, and fecal samples were collected daily from 4 to 15 days post-infection (dpi). Fecal oocyst shedding was quantified using the sucrose flotation method. Cecal tissues were collected at 5 dpi for histopathological analysis and lesion scoring. The animals in the 5-ALA group exhibited significantly greater weight gain and milder clinical signs than those in the control group. Fecal oocyst shedding was highest at 7 dpi in both groups; however, the 5-ALA group exhibited significantly lower oocyst output than the control group. The total number of fecal oocysts shed during the acute infection period was significantly lower in the 5-ALA group than in the control group. Histopathological analysis revealed that although both groups exhibited epithelial hyperplasia and *E. tenella* schizonts in the cecal submucosa, inflammatory cell infiltration, cecal tissue damage, and histological lesion scores were significantly lower in the 5-ALA group than in the control group. These results suggest that 5-ALA supplementation may mitigate the clinical, parasitological, and histological effects of *E. tenella* infection in broiler chickens.

Key words: 5-aminolevulinic acid, avian coccidiosis, broilers

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Introduction

Avian coccidiosis is caused by protozoan parasites of the genus *Eimeria* and poses an economic burden to the poultry industry worldwide, with estimated annual losses of USD 3 billion due to intestinal damage, impaired nutrient utilization, reduced feed conversion efficiency, and increased mortality[1,2]. Among the *Eimeria* species infecting chickens, *Eimeria tenella* is one of the most pathogenic and a major cause of severe cecal coccidiosis. Control measures rely on live vaccines and in-feed anticoccidial drugs. However, their prophylactic efficacy has been compromised by the widespread emergence and dissemination of drug-resistant parasite strains and by growing pressure to reduce routine chemoprophylactic use[3]. Although novel vaccines and alternative control strategies have been proposed, their large-scale application is limited by high costs, the risk of virulence reversion, and variable efficacy among strains. In addition, increasing consumer demand for antibiotic-free poultry production has intensified the search for safe and sustainable solutions[1,2]. Therefore, the development of innovative control strategies is essential to overcome drug resistance and reduce environmental contamination associated with anticoccidial use.

5-Aminolevulinic acid (5-ALA), an intermediate in heme biosynthesis, is synthesized from succinyl-CoA and glycine[4] and plays a critical role in cellular respiration and energy metabolism[5]. Previous studies in chickens have demonstrated that dietary supplementation with 5-ALA improves growth performance and modulates inflammatory responses to pathogens[6,7].

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In addition, 5-ALA attenuates toxin-induced disruption of the gut microbiota in zebrafish, and 5-ALA-mediated photodynamic therapy has shown antibiofilm activity against *Staphylococcus aureus*[8,9]. Dietary supplementation with 5-ALA enhanced the growth and immune function of broiler chickens[6]. Moreover, 5-ALA modulates the immune response in ruminants[10,11]. We reported that 5-ALA supplementation reduced fecal oocyst shedding and cecal inflammation in *E. tenella*-infected laying hens[12]. However, its effects on fast-growing broiler chickens, which have distinct physiological requirements, remain unclear. This study aimed to investigate the effects of 5-ALA supplementation on body weight gain, fecal oocyst shedding, and cecal histopathology in broiler chickens infected with *E. tenella*.

Materials and Methods

Parasite and Animals

A virulent *E. tenella* OPU strain maintained at the Laboratory of Animal Physiology at Okayama University (Okayama, Japan) was used. *E. tenella* oocysts were purified using the sugar flotation method, sporulated at 28 °C in 2.5% potassium dichromate, and stored at 4 °C.

Female 1-day-old broiler chicks (Ross 308, n = 90) were purchased from Fukuda Breeder Co. (Okayama, Japan). Female broilers were used because they show less variation in body weight gain and less aggressive behavior, thereby improving experimental consistency. Animal infection experiments were performed independently five times under identical conditions, with each trial consisting of 18 broiler chickens (nine controls and nine 5-ALA-treated birds), yielding a total sample size of 90 animals (45 per group).

The chickens were maintained in a coccidia-free room and provided with food and water *ad libitum*. The animals were housed in wire-floored individual cages to prevent coprophagy and cross-contamination between groups. The room temperature was maintained at 33 °C ± 2 °C under a 12 h light/12 h dark cycle. A commercial starter diet free of antibiotics and anticoccidial agents was provided throughout the experimental period.

Experimental design

5-ALA was obtained from Neopharma Japan Co., Ltd. (Tokyo, Japan). At 7 days post-hatching, the chickens were randomly assigned to either a control group (n = 9 per trial) or a 5-ALA-supplemented group (20 mg 5-ALA/kg diet, n = 9 per experiment). The chickens in both groups were orally inoculated with sporulated *E. tenella* oocysts (2.0×10^3 oocysts per animal) at 14 days of age. Three chickens were randomly selected and humanely euthanized in accordance with institutional animal care guidelines, and ceca were collected at 5 days post-infection (dpi) for histopathological analysis, as this point corresponds to a period of marked second-generation schizont maturation and severe cecal lesions due to *E. tenella* infection[13]. Each cecum was separated into three distinct regions (proximal, medial, and distal). Each tissue sample was fixed in 10% formaldehyde for histopathological observation.

Fecal collection and oocyst counting

The chickens were weighed every week on days 0, 7, 14, 21, and 28. After infection, the animals were observed daily for bloody stools, depression, ruffled feathers, and huddling, all of which are clinical signs of coccidiosis. Feces were collected daily from individual cages and pooled by treatment group from 5 to 15 days post-infection (dpi) for oocyst counting, as previously described[14]. Oocysts per gram of feces (OPG) were counted using the fecal flotation method with a saturated sucrose solution. Briefly, fecal samples (2 g/tube) were thoroughly mixed with 10 mL of distilled water, then centrifuged at 2,500 rpm for 5 min at room temperature. The supernatant was discarded, and 10 mL of saturated sucrose solution was added to the tubes. The tubes were mixed thoroughly and centrifuged at 2,500 rpm for 5 min at room temperature. The supernatant was transferred to 15 mL centrifuge tubes and mixed thoroughly. Then, 10 µL of the supernatant was placed onto a glass slide and covered with coverslip. Oocysts were counted in three replicate aliquots from each tube using light microscopy.

Histopathological analysis

The medial region of the cecum at 5 dpi was fixed in 10% formaldehyde, embedded in paraffin, and sliced into 6-µm-thick sections. The sectioned specimens were stained with hematoxylin and eosin (six specimens per chicken, at 200 µm intervals) and examined using a light microscope (Nikon Eclipse Ci-L plus, Nikon Solutions Co., Ltd., Tokyo, Japan) to assess lesions and parasite burden (eight fields per specimen at ×200 and ×400 magnification). The lesion and parasite burden scores were evaluated on a 0–4 scale as previously described[15,16]. Two independent researchers with histopathological expertise, blinded to treatment allocation, evaluated the slides.

Statistical analysis

Continuous variables were compared using Student's *t*-test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. Data are presented as the mean ± standard error of the mean. P-values of less than 0.05 were considered statistically significant. Individual chickens were considered experimental units. Data from five independent experiments were pooled for analysis after confirming that the direction of group differences was consistent across trials.

Results and Discussion

Clinical signs and Body weight

Clinical signs, including huddling, bloody stools, and depression, were observed in both groups at 4–6 dpi. However, these symptoms were milder and resolved earlier in the 5-ALA group (data not shown). There were no significant differences in body weight between the two groups before *E. tenella* infection at 14 days of age. However, after infection, body weight at 21 days (7 dpi) was 388.0 ± 9.6 g in the 5-ALA group and 292.0 ± 19.2 g in the control group. At 28 days (14 dpi), body weight was significantly higher in the 5-ALA group than in the control group (710.0 ± 7.1 g vs. 508.0 ± 19.9 g, $p < 0.05$) (Fig. 1).

The higher body weight in the 5-ALA group is consistent with

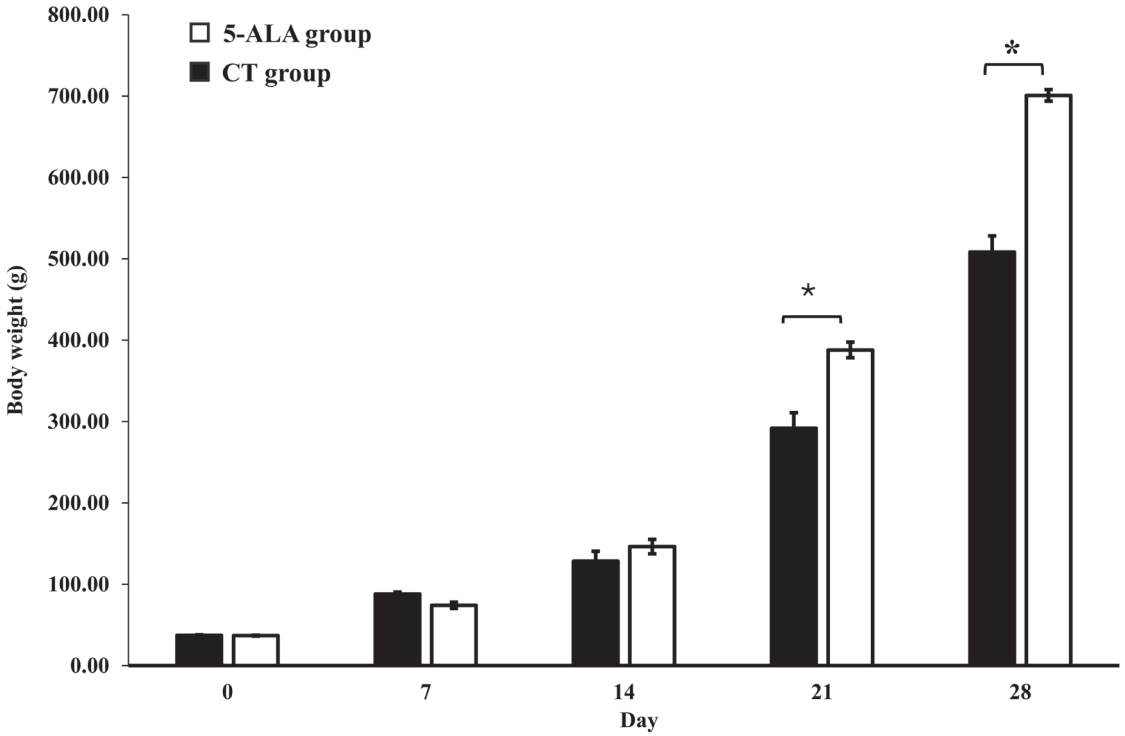

Fig. 1. **The body weight of broiler chickens was monitored weekly from 0 to 4 weeks of age.** Data were analyzed using Student's *t*-test and are presented as mean ± standard error of the mean. *$p < 0.05$, n = 9 per group.

previous studies showing that 5-ALA improves growth performance in stressed broilers[6]. Collectively, these results suggest that 5-ALA supplementation may help preserve intestinal integrity and improve recovery from *E. tenella* infection.

Fecal oocyst shedding

Fecal oocyst shedding began at 6 dpi. Oocyst output was highest at 7 dpi in both groups. The maximum number of oocysts was $3.93 \times 10^5 \pm 2.16 \times 10^4$ OPG in the 5-ALA group and $2.70 \times 10^6 \pm 8.79 \times 10^5$ OPG in the control group (Fig. 2A). The total oocyst output was significantly lower in the 5-ALA group ($1.04 \times 10^6 \pm 8.90 \times 10^4$ vs. $4.44 \times 10^6 \pm 7.14 \times 10^5$) (Fig. 2B). This observation is consistent with previous studies demonstrating that amino acid supplementation can mitigate intestinal damage caused by *Eimeria* spp[17]. Although methionine was not administered in the present study, previous studies have shown that sulfur-containing amino acids contribute to immune function and cellular homeostasis[18,19]. These findings suggest that nutritional support may enhance host resistance to coccidiosis. In the present study, 5-ALA supplementation reduced oocyst shedding in infected broilers. In contrast, our previous investigation in laying hens revealed that 5-ALA supplementation reduced oocyst shedding at 5 and 15 dpi. However, the total oocyst output during the first infection period did not differ significantly between the groups[12]. This result may reflect physiological differences between broilers and laying hens, particularly regarding body weight and nutrient absorption capacity. These findings underscore the importance of host factors such as production type and genetic background in determining the efficacy of 5-ALA against coccidiosis. The protective effects of 5-ALA appeared to be more evident in broiler chickens than in laying hens, possibly due to differences in growth rate and nutrient partitioning. Further studies are warranted to clarify these host-dependent effects and establish optimal conditions for the application of 5-ALA in poultry production.

Histological analysis

Lesion scores and histopathological changes in the cecum of broiler chickens were evaluated at 5 dpi after *E. tenella* infection. The control group exhibited pathological changes, including massive mucosal erosion, elongated crypts, marked inflammatory cell infiltration, and numerous immature schizonts deeply embedded in the tissue. However, the 5-ALA group showed better preservation of the cecal mucosal structure, with reduced inflammatory cell infiltration, improved epithelial integrity, preservation of most crypts, and fewer observable intracellular schizonts and oocysts (Fig. 2C).

Lesion scores were significantly lower in the 5-ALA group than in the control group (2.33 ± 0.17 vs. 3.67 ± 0.17, $p < 0.05$) (Fig. 2D). Parasite burden scores were also significantly lower in the 5-ALA group than in the control group (2.50 ± 0.12 vs. 3.83 ± 0.09, $p < 0.05$) (Fig. 2E). The significantly lower lesion scores

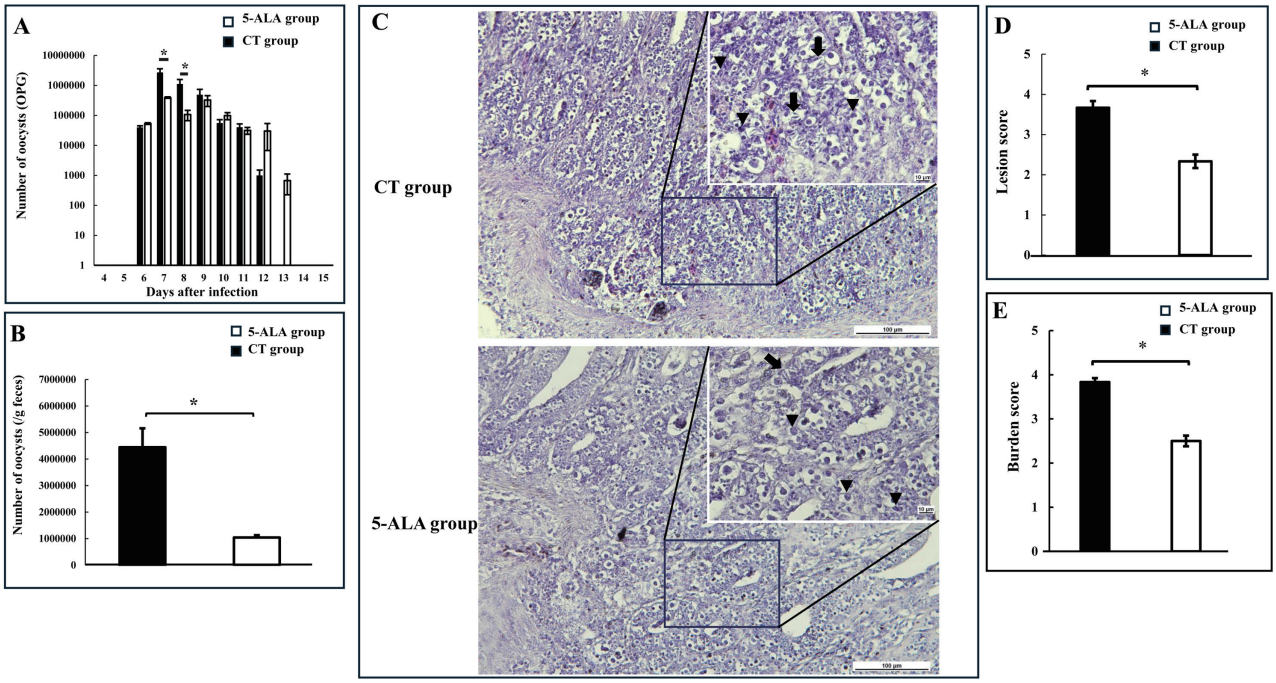

Fig. 2. **Fecal oocyst shedding and histopathological analysis.** A) Fecal oocyst shedding was measured daily from 5 to 15 days post-infection (dpi). B) Total number of oocysts in the feces. Open column: 5-aminolevulinic acid (5-ALA) group; filled column: control group. Data were analyzed using Student's *t*-test and are presented as mean ± standard error of the mean. *$p < 0.05$, n = 9 per group. C) Representative hematoxylin and eosin-stained cecal sections showing pathological changes at 5 dpi. Upper panels: control group at ×200 magnification (main image; scale bar = 100 μm) and ×400 magnification (inset; scale bar = 10 μm). Lower panels: 5-ALA group at ×200 magnification (main image; scale bar = 100 μm) and ×400 magnification (inset; scale bar = 10 μm). Insets show details of inflammatory infiltrates (black arrowhead) and intracellular schizonts (black arrow). D) Lesion scores. Data were analyzed using the Mann–Whitney U test and are presented as mean ± standard error of the mean. *$p < 0.05$, n = 9 per group. E) Parasite burden scores in the cecum of broiler chickens at 5 dpi. Data were analyzed using the Mann–Whitney U test and are presented as mean ± standard error of the mean. *$p < 0.05$, n = 9 per group.

in the 5-ALA group support the histopathological observations of reduced epithelial damage and inflammatory responses. In addition, fewer intracellular schizonts were observed in the 5-ALA group, indicating a reduced parasite burden, which was associated with lower inflammatory cell infiltration and reduced lesion severity. Histological analysis showed less tissue damage and lower lesion scores in the 5-ALA group at 5 dpi. These results indicate that 5-ALA supplementation helps preserve intestinal integrity during an *E. tenella* challenge. Maintenance of epithelial architecture is crucial for nutrient absorption and barrier function, both of which are severely compromised during *E. tenella* infection. Thus, the reduction in lesion severity induced by 5-ALA may enhance host resilience against infections. Although the present study did not investigate the underlying molecular mechanisms, previous reports have suggested that 5-ALA may modulate host inflammatory and oxidative stress responses[7] and support innate immune cell function[5], which may have contributed to the lower lesion severity observed in the present study.

In conclusion, this study demonstrated that dietary supplementation with 20 ppm 5-ALA markedly reduced cecal histopathological changes and growth depression caused by *E. tenella* infection in broiler chickens. These findings suggest that 5-ALA may support intestinal health and resilience during coccidial challenges in poultry. Nonetheless, further studies under different production conditions are warranted to support the practical application of 5-ALA in poultry production

Ethical approval

All procedures were reviewed and approved by the Animal Care and Use Committee of Okayama University (OKU-2022861) and were conducted strictly in accordance with the university's Policy on the Care and Use of Laboratory Animals.

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Author Contributions

Taqi Ahmad Hanif drafted the manuscript, collected and analyzed the data, and conducted the animal experiments. Toshimitsu Hatabu planned and oversaw the research, analyzed the findings, and edited the manuscript. Makoto Matsubayashi maintained the parasites. All authors approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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