

Effects of Coated Acidifiers on Growth Performance, Gastrointestinal Environment, and Serum Biochemical Indices in Yellow-Feathered Broilers (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different doses of coated acidifiers on growth performance, gastrointestinal environment, and serum biochemical indices in yellow-feathered broilers. A total of 300 healthy 1-day-old yellow-feathered broilers with similar body weight were selected and randomly allocated into 4 groups, with 5 replicates per group and 15 birds per replicate. The control group was fed a basal diet, while the experimental groups were fed basal diets supplemented with 0.1%, 0.3%, and 0.5% coated acidifiers, respectively. The experimental period lasted 63 days. The results showed that: 1) The final body weight and body weight gain in the 0.1%, 0.3%, and 0.5% coated acidifier groups were extremely significantly higher than those in the control group ($P < 0.01$). 2) The breast muscle percentage and leg muscle percentage in the 0.3% coated acidifier group were significantly higher than those in the control group ($P < 0.05$); the duodenal pH in the 0.3% coated acidifier group and the proventricular pH in the 0.5% coated acidifier group were significantly lower than those in the control group ($P < 0.05$); the duodenal trypsin and amylase activities and cecal lactic acid bacteria count in the 0.3% coated acidifier group were significantly higher than those in the control group ($P < 0.05$), while the *Escherichia coli* count was significantly lower than that in the control group ($P < 0.05$). 3) The alkaline phosphatase (AKP) activity in the 0.1% coated acidifier group was significantly higher than that in the control group, whereas the urea nitrogen content was significantly lower than that in the control group ($P < 0.05$); the AKP and catalase (CAT) activities and total antioxidant capacity in the 0.3% coated acidifier group were significantly higher than those in the control group ($P < 0.05$). In conclusion, dietary supplementation with coated acidifiers can improve growth performance, reduce intestinal

pH, enhance digestive enzyme activities, improve microbial flora composition, and optimize serum biochemical indices in yellow-feathered broilers, with an appropriate supplementation level of 0.3%.

Full Text

Effects of Coated Acidifier on Growth Performance, Gastrointestinal Environments and Serum Biochemical Indices of Yellow Broilers

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different doses of coated acidifier on growth performance, gastrointestinal environments, and serum biochemical indices of yellow broilers. Three hundred 1-day-old healthy yellow-feathered broilers with similar body weight were randomly allocated into four groups, each consisting of five replicates with 15 birds per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 0.1%, 0.3%, and 0.5% coated acidifier, respectively. The trial lasted for 63 days. The results showed: 1) The final body weight and body weight gain in the 0.1%, 0.3%, and 0.5% coated acidifier groups were significantly higher than those in the control group ($P < 0.01$). 2) The breast meat percentage and thigh meat percentage in the 0.3% coated acidifier group were significantly higher than those in the control group ($P < 0.05$). The duodenal pH in the 0.3% coated acidifier group and the glandular stomach pH in the 0.5% coated acidifier group were significantly lower than those in the control group ($P < 0.05$). The activities of trypsin and amylase in the duodenum and the number of lactobacilli in the cecum in the 0.3% coated acidifier group were significantly higher than those in the control group ($P < 0.05$), while the number of *E. coli* was significantly lower ($P < 0.05$). 3) The alkaline phosphatase (AKP) activity in the 0.1% coated acidifier group was significantly higher than that in the control group, while the urea nitrogen content was significantly lower ($P < 0.05$). The AKP, catalase (CAT) activities, and total antioxidant capacity in the 0.3% coated acidifier group were significantly higher than those in the control group ($P < 0.05$). In conclusion, dietary supplementation with coated acidifier can improve the growth performance of yellow broilers, reduce intestinal pH, enhance digestive enzyme activities, optimize microbial flora composition, and improve serum biochemical indices, with an optimal addition level of 0.3%.

Keywords: yellow broilers; growth performance; digestive enzyme; microbial

flora; serum biochemical indices

Introduction

Since antibiotics were discovered to promote animal growth, they have been widely used in livestock production, with some applications constituting outright abuse. As public concern over food safety has grown, the search for antibiotic alternatives has become a research priority. Acidifiers, classified alongside flavoring agents, enzyme preparations, and probiotics as health-promoting feed additives, are characterized by no residues, no drug resistance, and no toxicity, leading to their increasingly widespread application in feed. Acidifiers can reduce the pH of feed [1] and the digestive tract [2], promote animal feed intake [3], enhance digestive enzyme activities [4-5], and improve intestinal microbial flora [6-7]. Coated acidifiers utilize lipid-based slow-release technology to encapsulate the acidifier. Compared with compound acidifiers, coated acidifiers can improve product efficacy, release in the gastrointestinal tract, and provide more persistent and effective action.

China's yellow-feathered broilers are primarily imitation native chicken types, featuring high growth performance, strong stress resistance, attractive appearance, good meat quality, and the characteristic "three-yellow" phenotype, making them suitable for intensive farming with significant market competitive advantages. Poultry have relatively short digestive tracts, with feed passing quickly through the intestines. Particularly in young chicks, the digestive system is underdeveloped, gastric acid secretion is insufficient, and digestive enzyme activation is inadequate [8], resulting in low feed digestion and utilization rates. Therefore, adding substances that promote digestion and absorption to their diets is of great significance for their growth.

Current literature contains limited reports on the dosage and effects of coated acidifiers in yellow-feathered broilers. This study investigated the regulatory effects of coated acidifiers on growth performance, gastrointestinal environments, and serum biochemical indices of yellow broilers, exploring the impacts of different supplementation levels to provide a theoretical basis for using coated acidifiers to improve digestive function and promote growth in broiler production.

1.1 Experimental Design

Three hundred 1-day-old yellow-feathered broilers provided by Putian Guangdong Wens Poultry Co., Ltd. were randomly divided into four groups, with five replicates per group and 15 birds per replicate. There were no significant differences in body weight among replicates ($P > 0.05$). The control group was fed a basal diet without coated acidifier, while the experimental groups were fed

the basal diet supplemented with 0.1%, 0.3%, and 0.5% coated acidifier, respectively. The coated acidifier was provided by Mailun (Xiamen) Biotechnology Co., Ltd. (containing citric acid, lactic acid, phosphoric acid, etc., with 30% lactic acid and 48.6% total acid per gram of product). The trial lasted 63 days. The experimental diets were corn-soybean meal-based, formulated according to Chinese yellow-feathered broiler feed standards. The composition and nutrient levels of the basal diet are shown in . Birds were raised in cages under 24-hour lighting with free access to feed and water. Ventilation was provided by exhaust fans, and conventional immunization programs were followed.

1.2 Sampling and Measurements

1.2.1 Sampling Prior to sampling, broilers were fasted for 12 hours. On day 63 of the experiment, three healthy broilers with medium body weight were randomly selected from each replicate. Blood (5 mL) was collected from the wing vein and allowed to clot for 2 hours before serum was prepared and stored at -20 °C for serum biochemical analysis. After blood collection, broilers were slaughtered by neck bleeding. Contents from the crop, glandular stomach, muscular stomach, duodenum, jejunum, and ileum were collected for pH determination. Additional duodenal contents were collected for digestive enzyme activity assays. Both ceca were collected for microbial culture. Finally, slaughter performance was measured.

1.2.2 Growth Performance Broilers were weighed at 08:00 on days 1 and 63 by replicate. Daily feed supply, residual feed, and wastage were recorded to calculate body weight gain and feed-to-gain ratio. Mortality was recorded daily to calculate survival rate using the formula: Survival rate (%) = [(Number of birds housed - Number of deaths) / Number of birds housed] × 100.

1.2.3 Slaughter Performance Slaughter performance indices including dressing percentage, semi-eviscerated percentage, eviscerated percentage, breast meat percentage, and thigh meat percentage were measured according to the methods described by Yang Ning [9].

1.2.4 Intestinal pH Measurement Intestinal pH was measured using a PHS-3C pH meter.

1.2.5 Duodenal Digestive Enzyme Activity and Cecal Microbial Culture Duodenal amylase and trypsin activities were measured using kits purchased from Nanjing Jiancheng Bioengineering Institute. Cecal microbial culture was performed according to the method of Chen Jiexiang et al. [10]. *E. coli* was cultured using Beijing Luqiao MacConkey agar medium, and lactobacilli were cultured using Beijing Luqiao MRS medium.

1.2.6 Serum Biochemical and Antioxidant Indices Serum biochemical indices including total protein, albumin, urea nitrogen, and alkaline phosphatase were measured using kits (Shanghai Rongsheng Biological Pharmaceutical Co., Ltd.). Total protein was determined by the biuret method, albumin by the bromocresol green method, urea nitrogen by the urease continuous monitoring method, and alkaline phosphatase activity by the AMP method. Antioxidant capacity indices including malondialdehyde (MDA), catalase (CAT), and total antioxidant capacity (T-AOC) were measured using kits (Nanjing Jiancheng Bioengineering Institute). MDA content was determined by the thiobarbituric acid (TBA) method, while CAT activity and T-AOC were determined by visible light methods.

1.3 Data Processing and Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 16.0 statistical software. Multiple comparisons of means were performed using the LSD method. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$. Results are expressed as means \pm standard deviation.

2.1 Effects of Coated Acidifier on Growth Performance of Yellow Broilers

As shown in , there were no significant differences in initial body weight among groups ($P > 0.05$), all averaging approximately 38.0 g. The final body weight and body weight gain in the 0.1%, 0.3%, and 0.5% coated acidifier groups were significantly higher than those in the control group ($P < 0.01$), with final body weight increasing by 10.54%, 12.92%, and 10.24%, respectively, and body weight gain increasing by 10.80%, 13.17%, and 10.45%, respectively. There were no significant differences in feed-to-gain ratio or survival rate among groups ($P > 0.05$).

2.2 Effects of Coated Acidifier on Slaughter Performance of Yellow Broilers

As shown in , the breast meat percentage and thigh meat percentage in the 0.1%, 0.3%, and 0.5% coated acidifier groups were improved compared with the control group. Breast meat percentage increased by 4.79% ($P > 0.05$), 6.64% ($P < 0.05$), and 3.38% ($P > 0.05$), respectively, while thigh meat percentage increased by 3.42% ($P > 0.05$), 5.44% ($P < 0.05$), and 3.01% ($P > 0.05$), respectively. There were no significant differences in dressing percentage, semi-eviscerated percentage, or eviscerated percentage among groups ($P > 0.05$).

2.3 Effects of Coated Acidifier on Intestinal pH of Yellow Broilers

As shown in , the cecal pH in the 0.5% coated acidifier group was significantly lower than that in the control group ($P < 0.05$), decreasing by 4.97%. The duodenal pH in the 0.3% coated acidifier group was significantly lower than that in the control group ($P < 0.05$), decreasing by 1.99%. There were no significant differences in pH among the remaining intestinal segments ($P > 0.05$).

2.4 Effects of Coated Acidifier on Duodenal Digestive Enzyme Activity and Cecal Microbial Number of Yellow Broilers

As shown in , the activities of duodenal amylase and trypsin in the 0.1%, 0.3%, and 0.5% coated acidifier groups were improved compared with the control group. Amylase activity increased by 6.00% ($P > 0.05$), 14.00% ($P < 0.05$), and 4.00% ($P > 0.05$), respectively, while trypsin activity increased by 3.75% ($P > 0.05$), 8.86% ($P < 0.05$), and 3.43% ($P > 0.05$), respectively. The number of *E. coli* in the cecum of the 0.3% coated acidifier group was significantly reduced compared with the control group ($P < 0.05$), decreasing by 4.70%, while the number of lactobacilli was significantly increased by 3.67% ($P < 0.05$). There were no significant differences among the other groups ($P > 0.05$).

2.5 Effects of Coated Acidifier on Serum Biochemical Indices and Antioxidant Function of Yellow Broilers

As shown in , compared with the control group, the serum alkaline phosphatase activity in the 0.1% and 0.3% coated acidifier groups was significantly increased ($P < 0.05$) by 15.36% and 20.00%, respectively, while urea nitrogen content was significantly decreased ($P < 0.05$) by 11.86% and 13.56%, respectively. There were no significant differences in serum total protein or albumin content among groups ($P > 0.05$).

Compared with the control group, the serum CAT activity, T-AOC, and MDA content in the 0.1%, 0.3%, and 0.5% coated acidifier groups were improved. Specifically, CAT activity increased by 10.53% ($P > 0.05$), 19.43% ($P < 0.05$), and 17.00% ($P < 0.05$), respectively; T-AOC increased by 4.77% ($P > 0.05$), 11.85% ($P < 0.05$), and 4.08% ($P > 0.05$), respectively; and MDA content decreased by 12.50% ($P > 0.05$), 16.19% ($P < 0.05$), and 9.94% ($P > 0.05$), respectively.

3.1 Effects of Coated Acidifier on Growth Performance of Yellow Broilers

The addition of coated acidifier in this experiment promoted the improvement of body weight and body weight gain in yellow broilers, which is consistent with

reports by Khosravi et al. [11] and Abdel-Fattah et al. [12]. Numerous studies have shown that adding acidifiers to broiler diets can improve feed palatability, participate in metabolic reactions after entering the animal body, accelerate nutrient absorption and utilization, effectively inhibit the proliferation of harmful bacteria in the digestive tract, maintain microbial ecological balance, enhance immune function, and consequently improve broiler growth performance. Yin Jingdong et al. [13] reported that adding 0.5% citric acid to broiler diets increased weight gain and survival rate by 6.1% and 8.6%, respectively. Zhu Biquan et al. [14] reported that adding 1.5% fumaric acid, 1.5% citric acid, or 0.2% compound acidifier to broiler diets could improve growth performance to varying degrees, with 0.2% compound acidifier showing the best effect. This experiment obtained similar results, demonstrating that adding 0.3% coated acidifier to yellow broiler diets increased body weight by 12.92% and body weight gain by 13.17%, with superior effects compared to the 0.5% addition level.

3.2 Effects of Coated Acidifier on Slaughter Performance of Yellow Broilers

Slaughter performance indices provide the most intuitive comparison of meat yield in broilers, though few reports exist on the effects of acidifiers on slaughter performance. Ma Hongyan [15] demonstrated that adding 0.3% compound acidifier to broiler diets provided optimal weight gain effects and had some influence on slaughter performance, though differences among supplementation groups were not significant. Xu Gang [16] reported that adding acidifiers to broiler diets significantly increased semi-eviscerated percentage and eviscerated percentage by 3.89% and 4.11%, respectively. The current results are similar to these reports. Adding 0.3% coated acidifier to yellow broiler diets increased semi-eviscerated percentage, eviscerated percentage, breast meat percentage, and thigh meat percentage by 13.16%, 14.33%, 21.99%, and 20.62%, respectively. The 0.5% coated acidifier group showed increases of 10.48%, 11.34%, 14.93%, and 14.70%, respectively. Comparing slaughter performance, the 0.3% coated acidifier group demonstrated superior effects to the 0.5% group.

3.3 Effects of Coated Acidifier on Intestinal pH of Yellow Broilers

Literature reports on the effects of acidifiers on animal intestinal pH have yielded inconsistent results. Most studies indicate that acidifiers can reduce intestinal pH. Guo Xuefeng et al. [17] found that adding Kangtisuian and Ruweisuan to weaned piglet diets significantly reduced stomach and duodenal pH to highly significant levels. Chen Daiwen et al. [18] reported that adding 5 kg/t Lactobacillus preparation to piglet diets significantly reduced stomach and duodenal pH compared with the control group, without affecting other intestinal segments. Riskey et al. [19] found that adding 1.5% citric acid to corn-soybean meal piglet diets reduced jejunal pH from 6.76 to 6.69 and cecal pH from 6.36 to 6.19, though differences were not significant. Cao Zhihua et al. [20] detected

that adding 5, 10, and 15 g/kg organic compound acidifier to Avian broiler diets reduced glandular stomach pH with increasing concentration, though pH stabilized by day 5 in all groups. Cao Zhihua et al. [21] also reported no significant differences in jejunal pH among groups after adding different levels of compound acidifier to broiler diets. The current results showed that coated acidifier addition primarily affected duodenal pH, with the 0.3% group reducing duodenal pH by 1.99% compared with the control group, while other intestinal segments showed minimal changes.

3.4 Effects of Coated Acidifier on Duodenal Digestive Enzyme Activity and Cecal Microbial Number of Yellow Broilers

Changes in digestive tract pH significantly affect digestive enzyme activities. Zhu Xiaoping et al. [22] found that adding biological acidifier to early weaned piglet diets promoted gastric acid and pepsin secretion and increased amylase and trypsin activities. Guo Peng et al. [23] reported that adding 0.2% compound acidifier to broiler diets significantly increased duodenal amylase activity at 21 and 42 days of age. In this experiment, duodenal digestive enzyme activities in yellow broilers also changed after coated acidifier addition. Compared with the control group, the 0.5% coated acidifier group increased trypsin and amylase activities by 3.43% and 4.00%, respectively, while the 0.3% group increased them by 8.86% and 14.00%, respectively. These results are consistent with the aforementioned reports and may be related to the significant changes in duodenal pH mentioned previously.

Poultry have short colons, allowing digesta to quickly pass into the cecum, making the cecum an important site for microbial survival and activity [24]. The primary function of the indigenous cecal microflora is to prevent colonization by invading pathogenic microorganisms [25]. Acidifier supplementation reduces intestinal pH, destroying the suitable living environment for harmful bacteria while creating favorable conditions for beneficial bacteria such as lactobacilli. One possible pathway for improved amino acid and energy digestion and absorption is through reducing intestinal pathogen numbers, weakening competition between host nutrients and microbes, and decreasing endogenous losses [26]. Li et al. [27] reported that adding 0.5% organic acidifier B to 14-day-old weaned piglet diets reduced fecal *E. coli* numbers and increased lactobacilli numbers. Ma Hongyan et al. [28] demonstrated that compound acidifier supplementation improved the intestinal microbial environment in broilers by inhibiting *E. coli* growth and promoting lactobacilli proliferation, with *E. coli* numbers gradually decreasing and lactobacilli numbers gradually increasing as supplementation levels increased. The current results are similar, showing that coated acidifier supplementation reduced cecal *E. coli* numbers and increased lactobacilli numbers in yellow broilers, with the 0.3% group showing the best effects (lactobacilli increased by 3.67%, *E. coli* decreased by 4.70%).

3.5 Effects of Coated Acidifier on Serum Biochemical Indices and Antioxidant Function of Yellow Broilers

Changes in serum biochemical indices reflect alterations in cell permeability and metabolic function. Serum total protein has diverse physiological functions, primarily maintaining stable osmotic pressure and acid-base balance within blood vessels, transporting and regulating various metabolic substances, and maintaining normal immune function. Albumin is synthesized by the liver, and when liver function is impaired, albumin levels decrease in proportion to the severity of liver damage. In this experiment, serum total protein and albumin contents showed no significant changes, possibly because long-term coated acidifier supplementation allowed the body to regulate and adapt, weakening the acidifier's effects.

Serum alkaline phosphatase is a key metabolic enzyme with physiological roles in defense, immune regulation, and ion secretion [29]. The current results showed that coated acidifier supplementation increased alkaline phosphatase activity in yellow broilers of the same age, with the 0.3% group showing the best effect. Urea nitrogen is the main end product of protein metabolism, and serum urea nitrogen content is an important indicator of protein metabolism balance. Reduced urea nitrogen content indicates enhanced protein anabolism, improved nitrogen utilization, and promoted muscle growth [30]. In this experiment, serum urea nitrogen content in the coated acidifier groups was lower than in the control group, indicating that coated acidifier supplementation effectively promoted nutrient absorption and utilization, particularly protein, thereby optimizing growth performance in yellow broilers.

CAT plays an important physiological role in metabolizing reactive oxygen species (hydrogen peroxide, hydroxyl radicals, and superoxide anions) *in vivo* [31]. Few reports exist on the effects of acidifiers on serum CAT activity in broilers. This experiment showed that coated acidifier supplementation could increase serum CAT activity in yellow broilers to varying degrees, indicating that coated acidifiers can improve serum CAT activity to some extent.

The antioxidant defense system (antioxidant substances and enzymes) endows the body with considerable antioxidant damage capacity. T-AOC magnitude reflects the status of free radical metabolism in the body, and its determination is significant for evaluating enzymatic and non-enzymatic antioxidant capacity [32]. MDA, as a product of lipid peroxidation caused by free radicals attacking polyunsaturated fatty acids in biological membranes, reflects not only the rate and intensity of lipid peroxidation but also indirectly indicates the severity of body damage. The results showed that coated acidifier supplementation enhanced T-AOC and reduced MDA content, with the improvement in T-AOC demonstrating this effect.

In conclusion, dietary supplementation with coated acidifier can regulate intestinal pH, enhance digestive enzyme activities, optimize cecal microbial flora, improve serum physiological and biochemical indices, and consequently enhance

growth performance in yellow broilers, with an optimal addition level of 0.3%.

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