

Effects of Paraformic Acid Supplementation via Drinking Water on Growth Performance, Digestive Enzyme Activities and Cecal Microbiota in Broilers

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ABSTRACT

This study evaluated the effects of groundwater acidification with 0.05% paraformic acid (PFA) on growth performance, digestive enzyme activities, and cecal microbiota in broilers. Two hundred and forty one-day-old female broilers were assigned to two treatments, with six replicates of 20 birds each, and received either untreated groundwater (pH 7.87 ± 0.23) or groundwater supplemented with 0.05% PFA (final pH 3.38 ± 0.09) throughout the 40-day experimental period. On day 14, birds were sampled for assessment of fecal condition, gastrointestinal pH, digestive enzyme activities, and cecal microbiota. PFA supplementation did not significantly affect total water intake but significantly improved feed conversion ratio (FCR) and average daily gain (ADG) over the 40-day trial ($P < 0.05$). Birds receiving PFA showed visually lower fecal moisture and improved fecal consistency on day 14. PFA supplementation significantly increased pepsin activity in the proventriculus ($P < 0.05$). Cecal microbiota analysis revealed more unique operational taxonomic units and significant enrichment of *Lactobacillus* and *Anaerofustis* in the PFA group ($P < 0.05$). These findings indicate that supplementation of drinking water with 0.05% PFA can effectively lower groundwater pH without reducing water intake, while improving growth performance and modulating early digestive function and cecal microbial composition in broilers.

List of Abbreviations

FCR	: Feed Conversion Ratio
ADFI	: Average Daily Feed Intake
ADG	: Average Daily Gain
FBW	: Final Body Weight
IBW	: Initial Body Weight
OTU	: Operational Taxonomic Unit
PFA	: Paraformic Acid
TWI	: Total Water Intake
EPEF	: European Production Efficiency Factor

Keywords: Paraformic Acid, Drinking Water, Broilers, Digestive Enzymes, Cecal Microbiota

Introduction

The first two weeks after hatch are a critical period for establishment of digestive capacity and gastrointestinal microbial

communities in broilers. Drinking water is a practical route for early-life delivery of acidifying agents because exposure begins immediately after placement and can be adjusted independently of the diet [1-3].

Organic-acid-based water acidification has been investigated as a management strategy to modify water pH and gastrointestinal conditions, with reported effects on digestive enzyme activity and microbial ecology [2-7]. Paraformic acid (PFA; CAS No. 3081158-27-9) has been developed as a newer-generation formic acid derivative with characteristics including non-corrosiveness and non-irritancy [11]. Previous studies have shown that dietary supplementation with 0.1% PFA can improve growth-related outcomes and support intestinal and hepatic health in broilers [12]. However, limited information is available on the effects of PFA administered via drinking water on growth performance and gastrointestinal health in broilers.

Therefore, this study evaluated whether supplementation of groundwater with PFA affected water intake, growth

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performance, fecal condition, gastrointestinal pH, digestive enzyme activity, and cecal microbiota in broilers.

Review of Literature

Drinking-Water Acidification in Broilers

Drinking-water acidification is used in poultry production to alter the physicochemical environment of the water supply and, after ingestion, may influence conditions within the gastrointestinal tract. Hamid et al. reported that acidified drinking water affected gastrointestinal function in broilers, supporting the concept that water treatment can influence digestive physiology beyond simple modification of water pH [3]. More recent work has also examined commercial water acidifiers and mixed organic-acid preparations, although responses in growth performance and gut-health variables have not been uniform [4,6,7]. These differences indicate that efficacy cannot be generalized across acidifier products and should be evaluated in relation to formulation, dose, water source, and production environment.

Organic Acids and Digestive Function

A principal proposed mechanism of organic-acid supplementation is reduction of pH in the upper gastrointestinal tract. A more acidic environment favors conversion of pepsinogen to active pepsin and can therefore support the initial stages of protein digestion [2,3]. Van Immerseel et al. also described mechanistic effects of organic acids on enteric bacteria, emphasizing that antimicrobial activity depends on acid dissociation and interaction of undissociated acid molecules with bacterial cells [2]. In broilers, mixed organic acids administered through drinking water have been associated with changes in digestive capacity and gastrointestinal function [3,7].

Acidification and Cecal Microbiota

Early microbial colonization is dynamic and closely linked to development of the digestive tract. Guo et al. demonstrated marked spatial organization of gastrointestinal microbiota in broilers, illustrating that microbial responses should be interpreted in relation to intestinal location [1]. Dietary and water-based acidification strategies can modify cecal community composition, but the direction and magnitude of the response vary among studies [4-7]. *Lactobacillus* is a common lactate-producing genus in the chicken gastrointestinal tract, whereas *Anaerofustis* includes organisms capable of producing short-chain fatty acids such as acetate and butyrate [8]. Cross-feeding between lactate producers and short-chain-fatty-acid-producing bacteria is biologically plausible, although microbial metabolite concentrations must be measured directly before functional metabolic effects can be concluded [10].

Rationale for PFA-Based Water Acidification

Previous studies have shown that dietary supplementation with 0.1% PFA can improve growth-related outcomes and support intestinal and hepatic health in broilers [11,12]. However, evidence is limited regarding whether PFA can reduce groundwater pH while maintaining water intake and simultaneously influence digestive enzyme activity and cecal microbial composition. This evidence gap provided the rationale for the present 40-day study, which included targeted gastrointestinal and microbiota assessments on day 14. The study therefore evaluated both overall production performance and early digestive and microbial responses.

Materials and Methods

Two hundred and forty one-day-old female broilers were randomly assigned to two treatments, with six replicates of 20 birds per treatment. The control group received groundwater (pH 7.87 ± 0.23), whereas the treatment group received groundwater supplemented with 0.05% PFA (final pH 3.38 ± 0.09) for 6 h per day throughout the 40-day experimental period. Water intake and growth performance were recorded throughout the trial. On day 14, two broilers from each replicate (12 birds per treatment) were selected for sampling. Fecal consistency and diarrhea status were assessed 1 h after feeding. The proventriculus, gizzard, duodenum, and jejunum were collected for gastrointestinal pH determination, and pepsin, trypsin, and amylase activities were measured in the corresponding gastrointestinal samples. Cecal contents were collected for microbiota analysis using 16S rRNA high-throughput sequencing.

PFA (minimum purity, 70%; trade name: Megacid-FL) was provided by Numega Nutrition Sdn. Bhd., Malaysia.

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using SAS University Edition. Differences between group means were separated using Duncan's multiple range test. Results are presented as mean $\pm$ SEM, and different superscript letters within the same row indicate significant differences ($P < 0.05$), in accordance with the source dataset.

Results

Water Intake and Growth Performance are shown in Table 1. Over the 40-day experimental period, total water intake did not differ significantly between treatments. However, FCR and ADG differed significantly between the control and 0.05% PFA groups ($P < 0.05$). Thus, acidifying groundwater from pH 7.87 to 3.38 with 0.05% PFA did not reduce water consumption and was associated with improved feed conversion and daily gain.

Table 1: Effects of 0.05% PFA on Growth Performance and Water Intake of Broilers over the 40-Day Experimental Period (Mean $\pm$ SEM)

Items	Control	0.05% PFA
IBW (g/bird, 1 d)	45.65 $\pm$ 2.85	44.53 $\pm$ 1.89
ADFI (g/bird/day)	104.84 $\pm$ 5.18	110.22 $\pm$ 5.34
FBW (g/bird, day 40)	2637.48 $\pm$ 85.94b	2997.36 $\pm$ 94.88a
ADG (g/bird/day)	65.94 $\pm$ 2.62b	73.82 $\pm$ 2.47a
FCR	1.59 $\pm$ 0.65a	1.49 $\pm$ 0.37b
TWI (mL/bird)	7815.1 $\pm$ 150.25	7561.5 $\pm$ 124.78
EPEF	480	495

Note: Different superscript letters (a,b) within the same row indicate significant differences ($P < 0.05$).

Fecal Condition. On day 14, 1 h after feeding, birds in the control group showed visibly wetter feces and more wet spots on the pads, whereas birds receiving 0.05% PFA showed lower apparent fecal moisture and better fecal consistency (Fig 1A-1B). The feathers around the cloaca also appeared cleaner in the PFA group (Fig 2A-2B). Because these observations were not accompanied by a reported quantitative fecal-moisture or lesion-scoring analysis, they should be interpreted as descriptive findings.

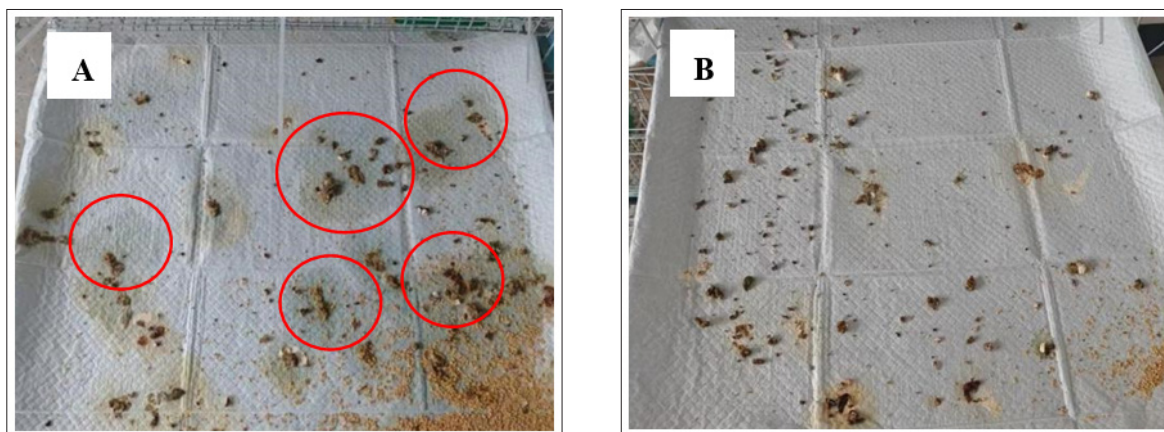


Figure 1: Fecal condition 1 h after feeding on day 14 (A: control; B: 0.05% PFA)

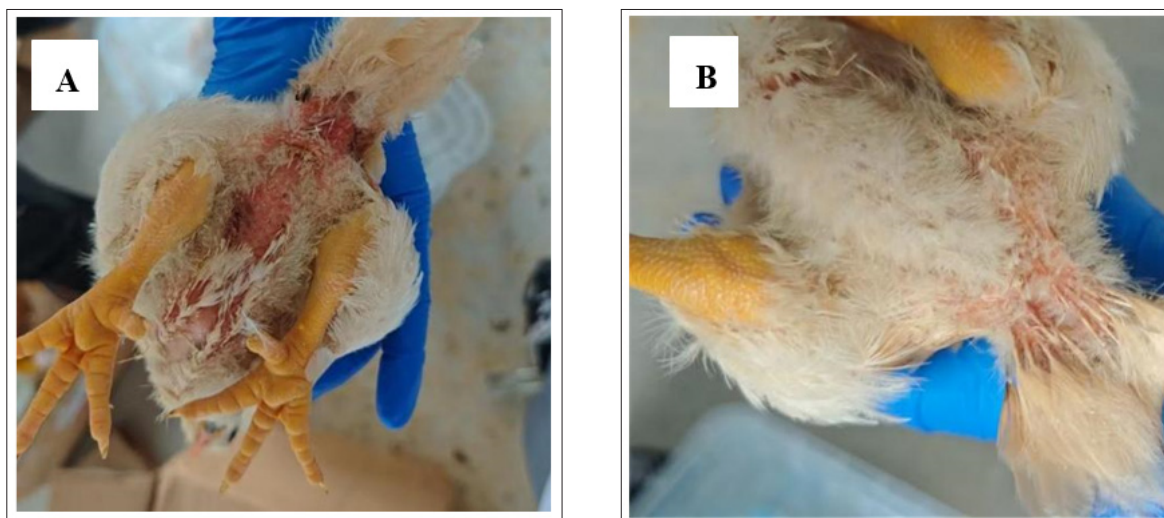
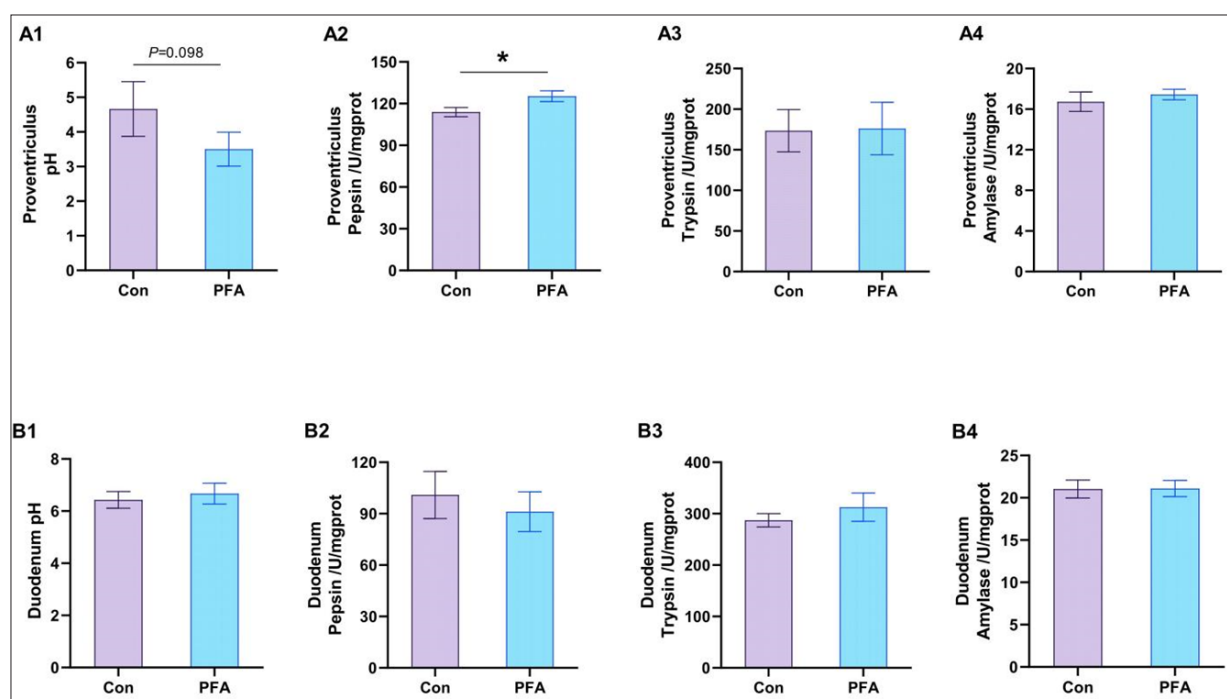


Figure 2: Cloacal feather condition on day 14 (A: control; B: 0.05% PFA)

Gastrointestinal pH and Digestive Enzyme Activities are presented in Figure 3. On day 14, drinking-water supplementation with 0.05% PFA significantly increased proventricular pepsin activity ($P < 0.05$), while proventricular pH showed a numerical decrease and jejunal pepsin activity showed a numerical increasing trend in the PFA group, but neither reached statistical significance.



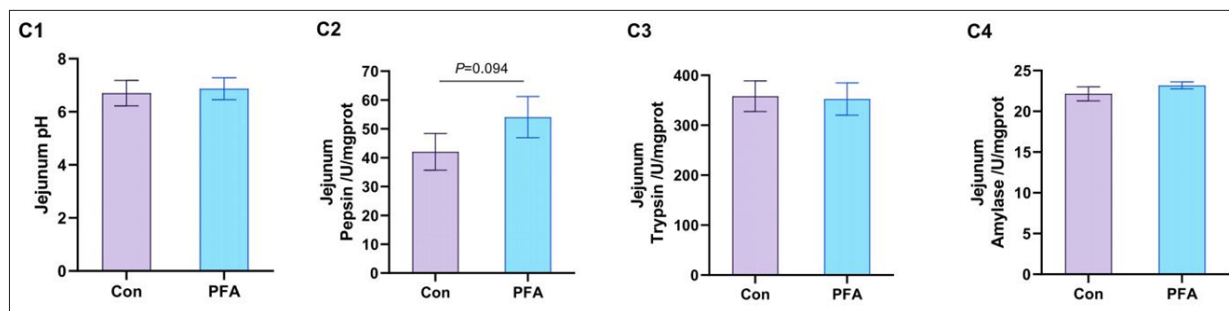


Figure 3: Effects of 0.05% PFA supplementation via drinking water on gastrointestinal pH and digestive enzyme activities in broilers at day 14. (A1–A4) Proventriculus: luminal pH, pepsin, trypsin, and amylase activities. (B1–B4) Duodenum: luminal pH, pepsin, trypsin, and amylase activities. (C1–C4) Jejunum: luminal pH, pepsin, trypsin, and amylase activities. Data are presented as mean ± SEM (n = 6). An asterisk “*” indicates a significant difference ($P < 0.05$)

Cecal Microbiota Composition. On day 14, the Venn diagram showed more unique OTUs in the 0.05% PFA group than in the control group (Fig 4). This descriptive result indicates treatment-associated differences in detected cecal taxa but, by itself, does not establish an increase in overall microbial diversity.

At the genus level (Figures 5–6), differential analysis showed significant enrichment of *Lactobacillus* and *Anaerofustis* in the PFA group ($P < 0.05$). *Lactobacillus* is associated with lactate production, whereas *Anaerofustis* includes taxa capable of producing short-chain fatty acids such as acetate and butyrate.

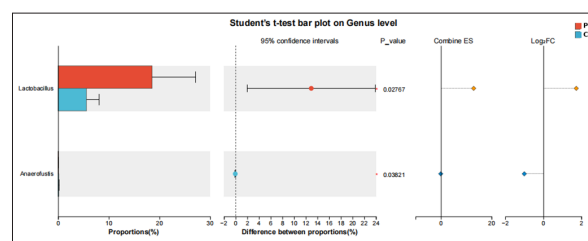


Figure 6: Genus-level comparison of cecal microbiota between groups

Discussion

Over the 40-day trial, supplementation with 0.05% PFA significantly improved FCR and ADG without reducing total water intake. This finding is consistent with previous research showing that drinking-water acidification can affect growth performance in broilers [4]. The response to acidification may vary with product composition, dose, diet, bird type, water characteristics, and production conditions [5,6]. In the present study, reducing drinking-water pH to 3.38 did not adversely affect water intake. This is important in practical application, as excessive acidification may reduce water palatability. On day 14, birds receiving PFA also showed visually improved fecal consistency. Dietary supplementation with PFA at 1,000 mg/kg has previously been reported to improve feed efficiency and affect hepatic inflammatory responses in broilers [12]. Together, these findings suggest that PFA may have beneficial effects on broiler performance when administered either through feed or drinking water, although the effective dose and mode of application differ between the two approaches.

PFA supplementation significantly increased pepsin activity in the proventriculus, while proventricular pH showed a numerical decrease and jejunal pepsin activity showed a numerical increase. Similar effects of organic-acid-based drinking-water additives on gastrointestinal pH and digestive enzyme activity have been reported in broilers [3,7]. A lower pH in the upper gastrointestinal tract favors the conversion of pepsinogen to pepsin, which may facilitate protein digestion [2]. However, nutrient digestibility was not measured, and further studies are needed to determine whether the observed changes in enzyme activity result in improved nutrient utilization.

PFA supplementation also altered the cecal microbial profile, with significant enrichment of *Lactobacillus* and *Anaerofustis*. *Lactobacillus* is a common lactate-producing genus in the chicken

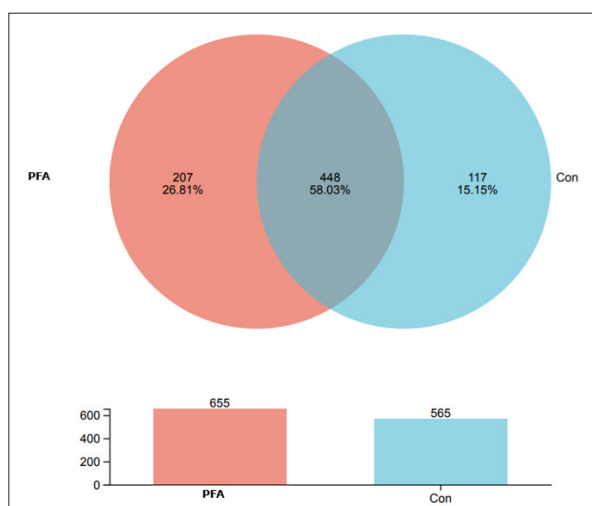


Figure 4: OTU-level Venn diagram of cecal microbiota on day 14

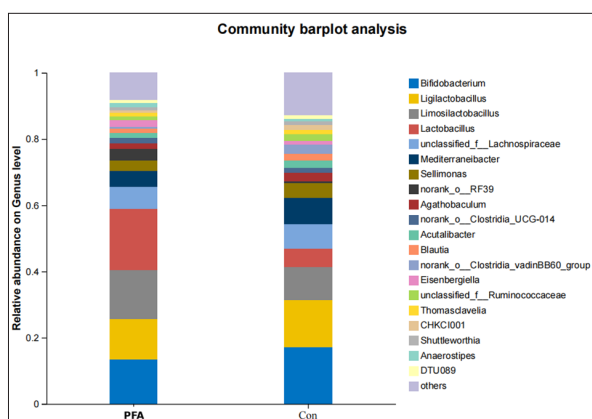


Figure 5: Genus-level relative abundance of cecal microbiota on day 14

gastrointestinal tract and plays an important role during early microbial colonization [1,7]. *Anaerofustis stercorihominis* has been reported to produce acetate and butyrate as fermentation end products, and changes in *Anaerofustis* abundance have also been reported in response to dietary interventions in broilers [8,9]. Interactions between lactate-producing bacteria and short-chain-fatty-acid-producing bacteria may contribute to changes in the intestinal microbial environment [10]. Previous research with dietary PFA also reported changes in cecal microbiota accompanied by improved intestinal development in broilers [11]. These findings are consistent with the present observation that PFA supplementation was associated with changes in cecal microbial composition.

Findings

Over the 40-day experimental period, acidifying groundwater with 0.05% PFA reduced water pH from 7.87 to 3.38 without reducing total water intake and significantly improved FCR and ADG.

At the day-14 sampling, PFA significantly increased pepsin activity in the proventriculus, while proventricular pH and jejunal pepsin activity showed numerical changes that did not reach statistical significance. Trypsin and amylase activities were not significantly affected. Cecal microbiota analysis identified more unique OTUs in the PFA group, with significant enrichment of *Lactobacillus* and *Anaerofustis*. Visual assessment also indicated drier feces and cleaner cloacal feathers in birds receiving PFA.

Conclusions

Under the conditions of this 40-day study, supplementation of drinking water with 0.05% PFA reduced groundwater pH without affecting water intake and significantly improved FCR and ADG. At day 14, PFA supplementation significantly increased proventricular pepsin activity and altered the cecal microbial profile, while proventricular pH and jejunal pepsin activity showed numerical changes that did not reach statistical significance. These results indicate that PFA supplementation via drinking water can improve growth performance and influence digestive function and cecal microbial composition in broilers.

Recommendations

Further studies should evaluate different PFA concentrations and drinking-water qualities under commercial production conditions. Sampling at different production stages is also needed to determine whether the digestive-enzyme and cecal-microbiota responses observed on day 14 persist throughout the production cycle. Measurements of fecal moisture, nutrient digestibility, and short-chain fatty acid concentrations would provide additional information on the functional significance of these gastrointestinal and microbial changes. Comparative studies of drinking-water and dietary PFA supplementation may also help determine appropriate application strategies for broiler production.

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Conflict of Interest

The authors declare no conflict of interest.

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