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Impact of organic acid supplementation on gut health and post-weaning diarrhea in early-weaned piglets

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Abstract

Post-weaning diarrhea (PWD) caused by pathogenic *Escherichia coli* is a major challenge in pig production. This study evaluated the efficacy of dietary organic acids in mitigating PWD and enhancing performance in weanling pigs. Thirty piglets, weaned at (21 ± 2 days old), were allocated into control and treatment groups, with the latter receiving malic acid and medium-chain fatty acids (lauric acids) for 28 days. Supplementation markedly reduced post weaning diarrhea. Treated piglets exhibited improved hematological and biochemical parameters, reduced severity of diarrhea, and superior growth performance, including average daily gain and feed conversion ratio. These findings indicate that organic acids improve gut health, reduce pathogen load, and enhance productivity, providing a sustainable alternative to antibiotics for managing PWD.

Keywords: Early weaned piglets, Organic acid, Post weaning diarrhea, Supplementation

Introduction

The piggery sector is one of the most important components of global livestock production, contributing substantially to food security, rural livelihoods, and the global meat industry. The worldwide pig population is estimated at nearly 900 million, underscoring its significance as a source of high-quality animal protein. In India, pigs constitute about 1.7% of the national livestock population, with nearly half concentrated in the North-Eastern region, where pig farming serves as a vital source of nutrition and income for marginal and smallholder farmers. Despite its socio-economic importance, the sector faces persistent challenges arising from disease outbreaks and management stressors that compromise productivity and profitability. Among these, weaning stress is regarded as one of the most critical bottlenecks in swine production.

Traditionally, piglets are weaned at 5-7 weeks of age; however, intensive production systems often employ early weaning at 3-4 weeks to improve sow reproductive efficiency, reduce lactational stress, and increase annual litter frequency. While this strategy enhances overall productivity, it exposes piglets to considerable physiological, nutritional, and behavioral stressors. Separation from the sow, abrupt dietary transition from easily digestible milk to complex solid feed, exposure to new microbial populations, and regrouping stress collectively disturb gut homeostasis (Tang *et al.*, 2024) [19]. These disruptions impair digestive enzyme activity, weaken intestinal barrier integrity, alter the composition of gut microbiota, and predispose piglets to gastrointestinal disorders, most notably post-weaning diarrhea (PWD).

PWD is one of the most prevalent and economically devastating conditions in pig production, contributing to impaired growth performance, reduced feed efficiency, and increased morbidity and mortality in weanling pigs. The disease is mainly associated with pathogenic strains of *Escherichia coli* (*E. coli*), particularly enterotoxigenic *E. coli* (ETEC) strains expressing F4 (K88) and F18 fimbriae. These strains colonize the small intestine, adhere to specific epithelial receptors, and produce enterotoxins such as heat-stable (ST) and heat-labile (LT) toxins that disrupt electrolyte and fluid balance, increase intestinal permeability, and cause profuse watery diarrhea (Park *et al.*, 2025) [18]. Severe cases may lead to dehydration, shock, and sudden death. Furthermore, ETEC infection alters mucosal

immunity and impairs mucin (MUC2) production, thereby exacerbating gut inflammation and pathogen invasion (de Groot *et al.*, 2024)^[17].

For decades, the control of PWD has relied heavily on prophylactic and therapeutic antibiotics. While effective, their extensive use has contributed to the emergence of antimicrobial resistance, concerns of drug residues in pork, and environmental contamination. These challenges have intensified the need for sustainable and safe alternatives that can protect gut health and reduce pathogen load without compromising growth performance. Organic acids (OAs) have emerged as promising non-antibiotic feed additives with multifaceted effects on gut physiology and health. They include short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), and derivatives such as lactic, fumaric, malic, and benzoic acids. Their antimicrobial action is attributed to the ability of undissociated acids to penetrate bacterial cell membranes, dissociate in the cytoplasm, and disrupt intracellular pH balance and metabolism, thereby suppressing pathogens such as *E. coli* and *Salmonella* (Connolly *et al.*, 2025)^[16]. Additionally, OAs lower gastric pH, enhance protein digestion, delay gastric emptying, improve mineral bioavailability, and stimulate pancreatic enzyme activity. They also promote beneficial microbiota such as *Lactobacillus* spp., strengthen mucosal integrity, and modulate immune responses (Tang *et al.*, 2024; Antioxidants, 2025)^[19, 20].

Recent studies have demonstrated that OA supplementation can mitigate diarrhea, improve intestinal morphology, enhance nutrient digestibility, and support growth performance in post-weaning pigs. For instance, Tang *et al.* (2024)^[19] reported that organic acids regulate redox homeostasis and reinforce intestinal barrier function, while de Groot *et al.* (2024)^[17] highlighted their role in improving villus height, villus-to-crypt ratio, and cytokine modulation. Park *et al.* (2025)^[18] further demonstrated that supplementation with OAs and monoglycerides reduced diarrhea incidence, fecal shedding of pathogenic *E. coli*, and bacterial translocation in ETEC F18-challenged piglets. Similarly, Connolly *et al.* (2025)^[16] showed that propionic acid-preserved grain enhanced protein digestion and gut health in low-protein diets without compromising growth performance. A recent study published in *Antioxidants* (2025)^[20] also confirmed that OA blends improved growth, antioxidant status, intestinal morphology, and expression of defense-related genes in weanling pigs. Despite these advances, limited data are available under Indian production

systems, where environmental stressors, microbial load, and farm management practices differ markedly from those in temperate regions.

The present study was therefore designed to evaluate the protective role of dietary organic acids in early-weaned piglets under Indian production conditions. Specifically, the objectives were to (i) identify *E. coli* pathotypes associated with PWD in the study population, (ii) assess the efficacy of organic acid supplementation in reducing diarrhea incidence.

Materials and Methods

Experimental Site and Ethical Approval

The present investigation was carried out at the Swine Production Farm (SPF) of the Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh, India. The experimental station maintains standardized housing, feeding, and health management practices consistent with the Bureau of Indian Standards (BIS) and ICAR guidelines for swine husbandry. All experimental procedures, including animal handling, sample collection, and euthanasia (where applicable), were conducted strictly in accordance with the Institutional Animal Ethics Committee (IAEC) protocols, ensuring compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations for animal research in India.

Experimental Animals and Design

The study utilized apparently healthy crossbred piglets, weaned at (21 ± 2 days old) days of age, representing the early weaning phase known to induce substantial physiological and microbial stress (Campbell *et al.*, 2013). A total of 30 number of early-weaned piglets (21 ± 2 days old) of uniform body weight (6-7 kg) and comparable litter origin were selected and divided into 5 groups with 6 piglets in each group. The groups were comprised of 1 control group (group without any supplementation) and 4 treatment groups (supplemented with two different organic acids). Commercially available OAs, namely malic acid (MA) and Lauric acid were selected and procured based on thorough review of research articles ethnopharmacological reports. The OAs which possessing antimicrobial, antioxidant and gut modulating properties were included in the present study and orally administered at two different doses in the diet of piglets for 4 weeks.

Experimental plan of work

Experimental design				
Duration: 4 weeks post-weaning				
OAs used: Malic acid & Lauric acid				
Control group	Treatment group-1	Treatment group-2	Treatment group-3	Treatment group-4
Basal diet only	Malic acid (12g/kg feed)	Malic acid (24g/kg feed)	Lauric acid (7.5g/kg feed)	Lauric acid (15g/kg feed)
n=6	n=6	n=6	n=6	n=6
Sample collection: Blood/Serum/Faecal material				
Day of collection: -1, 7, 14, 28 of trial				

Randomization was performed using a completely randomized design (CRD), ensuring that litter and sex distribution were balanced across groups to minimize confounding variation (Kyriazakis & Whittemore, 2006)^[24]. The experimental feeding trial was conducted over a period of 28 consecutive days following weaning.

Housing and Management

All piglets were housed in environmentally controlled pens with concrete floors and slatted drainage, ensuring hygienic conditions. The ambient temperature (24-28 °C) and relative humidity (60-70%) were maintained using fans and foggers, replicating commercial swine farm conditions in India. Each

pen was equipped with stainless steel feeders and nipple drinkers to provide ad libitum access to feed and water. Pens were routinely cleaned and disinfected to reduce environmental pathogen load, a critical factor in minimizing background microbial contamination during post-weaning studies (Fairbrother *et al.*, 2005) [22].

Routine health care, including deworming and vaccination schedules, was followed in line with IVRI farm practices. Piglets were observed daily for signs of respiratory, enteric, or systemic illness, and animals showing unrelated disease conditions were excluded from statistical analysis.

Dietary Formulation and Organic Acid Supplementation

The basal diet was a corn-soybean meal formulation meeting the nutrient requirements of weaned pigs as per NRC (2012). Diets were isonitrogenous and isocaloric across groups.

The organic acid blend consisted of a) Malic acid (C4 dicarboxylic acid)- Selected for its role in energy metabolism and buffering capacity in the gastrointestinal tract (Mroz *et al.*, 1997); b) [9] Medium-chain fatty acids (Lauric acid)- Chosen due to their proven bactericidal activity against Gram-negative enteric pathogens such as *E. coli* and *Salmonella* (Devi & Kim, 2016; Zentek *et al.*, 2013) [2, 15].

The blend was incorporated into the diet at 0.5-1.0% inclusion levels, as determined from previous studies and pilot trials conducted under Indian field conditions. Diets were prepared weekly in small batches to maintain freshness and homogeneity, and feed samples were analyzed for proximate composition following AOAC (2016) methods.

Clinical Monitoring and Fecal Scoring

Daily clinical observations included feed intake, behavioral activity, hydration status, and fecal consistency. A fecal scoring system (0-3 scale) was adopted:

- 0 = normal, firm feces
- 1 = soft feces
- 2 = semi-liquid feces
- 3 = watery diarrhea

The scoring allowed quantitative monitoring of post-weaning diarrhea (PWD) incidence and severity (Tsiloyiannis *et al.*, 2001) [13]. Piglets with persistent watery diarrhea were closely monitored for dehydration, and oral rehydration therapy was provided if necessary to minimize mortality bias.

Sample Collection

Fecal Samples

Fresh fecal samples were collected directly from the rectum using sterile swabs on days 0, 7, 14, 21, and 28. Samples were transported immediately under chilled conditions (4 °C) to the Microbiology Laboratory, IVRI, for bacteriological and molecular assays.

Blood Samples

Jugular venipuncture was performed aseptically on days 0, 14, and 28. Approximately 5 mL of blood was collected per piglet, EDTA vials were used for hematological assays and Plain vials used for serum biochemical and enzymatic analysis. Samples were centrifuged at 3000 rpm for 15 minutes, and serum was stored at -20 °C until further analysis.

Hematological Analysis

Hematological parameters were determined using an automated hematology analyzer (Sysmex XT-2000iV, Japan). Parameters included hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), and total leukocyte count (TLC). These indices serve as indicators of oxygen-carrying capacity, hydration status, and systemic stress (Ferrara *et al.*, 2017) [4].

Serum Biochemical and Enzymatic Assays

Serum total protein, albumin, and glucose concentrations were measured using commercial diagnostic kits (Erba Mannheim, India) in a semi-automated biochemical analyzer. Serum amylase and lipase activities were assessed using kinetic assay kits (Randox, UK) as indicators of digestive efficiency. These biochemical markers reflect nutritional status and pancreatic enzyme activity under dietary interventions (Upadhaya *et al.*, 2014; Connolly *et al.*, 2025) [14, 16].

Growth Performance and Carcass Traits

Piglets were weighed individually on days 0, 14, and 28 to calculate:

- **Average daily gain (ADG)** = (Final BW - Initial BW)/Number of days
- **Average daily feed intake (ADFI)** = Feed consumed/Number of days
- **Feed conversion ratio (FCR)** = ADFI/ADG

At the end of the trial, ultrasound backfat thickness (uBFT) was measured at the P2 position (65 mm off the dorsal midline at the last rib) using a portable ultrasound scanner (SonoSite M-Turbo, USA). uBFT serves as a non-invasive indicator of fat deposition and carcass quality, reflecting the efficiency of nutrient utilization (Patel & Abate, 2013).

Statistical Analysis

All data were subjected to statistical analysis using SPSS v25.0 (IBM Corp., Armonk, USA). The normality of data distribution was confirmed using the Shapiro-Wilk test. Comparisons between groups were performed using Analysis of Variance (ANOVA) under a completely randomized design. Significant differences were determined at $p < 0.05$, and results are expressed as mean $\pm$ standard error of mean (SEM).

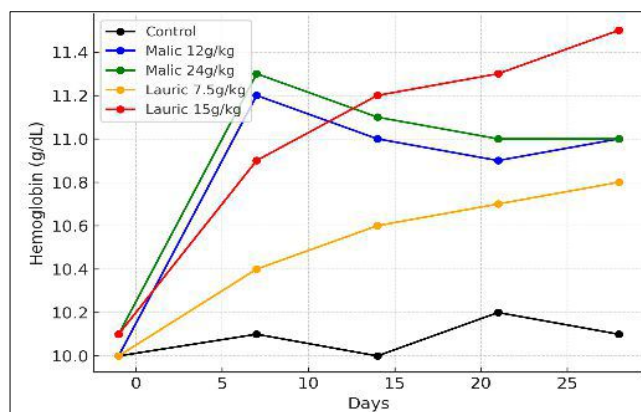
Where appropriate, repeated measures ANOVA was applied for longitudinal variables (fecal scores, hematology, and serum parameters across days). Post hoc comparisons were performed using Tukey's test to adjust for multiple comparisons.

Results and Discussion

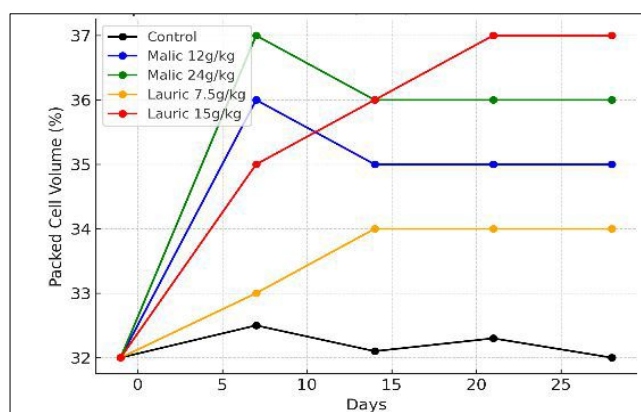
Hematological Parameters

Baseline values in the control group did not change significantly across the 28-day trial. In contrast, piglets supplemented with malic and lauric acids showed significant ($p < 0.05$) improvement in hemoglobin (Hb) and packed cell volume (PCV). When malic acid (12 g/kg) feed then Hb and PCV increased significantly from day -1 to day 7, stabilizing thereafter. Total erythrocyte count (TEC) and total leukocyte count (TLC) followed a similar trend. When malic acid (24 g/kg) feed, Similar improvements in Hb, PCV, TEC, and TLC were observed, with significance noted primarily during the first week. And then when lauric acid (

15 g/kg) feed. Marked improvements in Hb and PCV were sustained from day 14 to day 28, suggesting a dose-dependent effect.



1



2

Fig 1: Changes in Hemoglobin and PCV levels in control and treatment groups across the study period

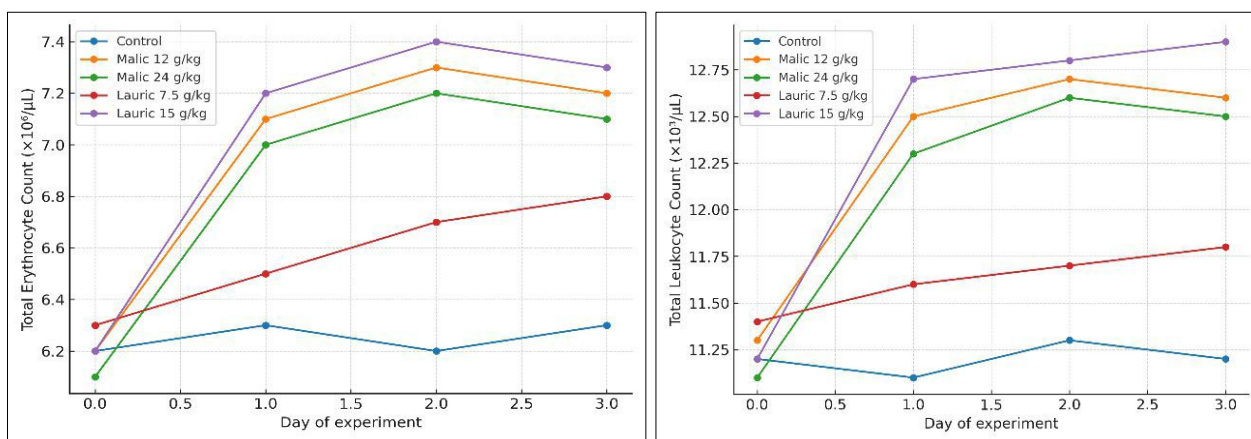


Fig 2: Changes in TEC and TLC across different dietary treatments

Serum Biochemical Profile

In the control group, no significant changes in total protein, albumin, glucose, amylase, or lipase were observed that the Glucose level Both malic acid groups (12 g/kg and 24 g/kg) showed significant increases from day -1 to day 7, with stabilization thereafter. Lauric acid at 15 g/kg also improved glucose significantly from day 14 to day 28. Total Protein &

Albumin have no significant changes across all groups (range: total protein 3.7-4.9 g/dL; albumin 2.7-3.4 g/dL). And the Amylase & Lipase have enzyme activities fluctuated within normal ranges (amylase: 1175-1875 IU/L; lipase: 24-42 IU/L) without significant differences among groups.

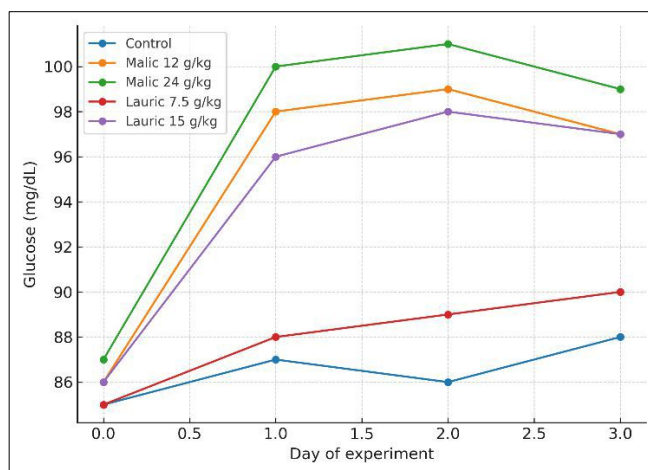


Fig 3: Serum glucose trends across treatment groups

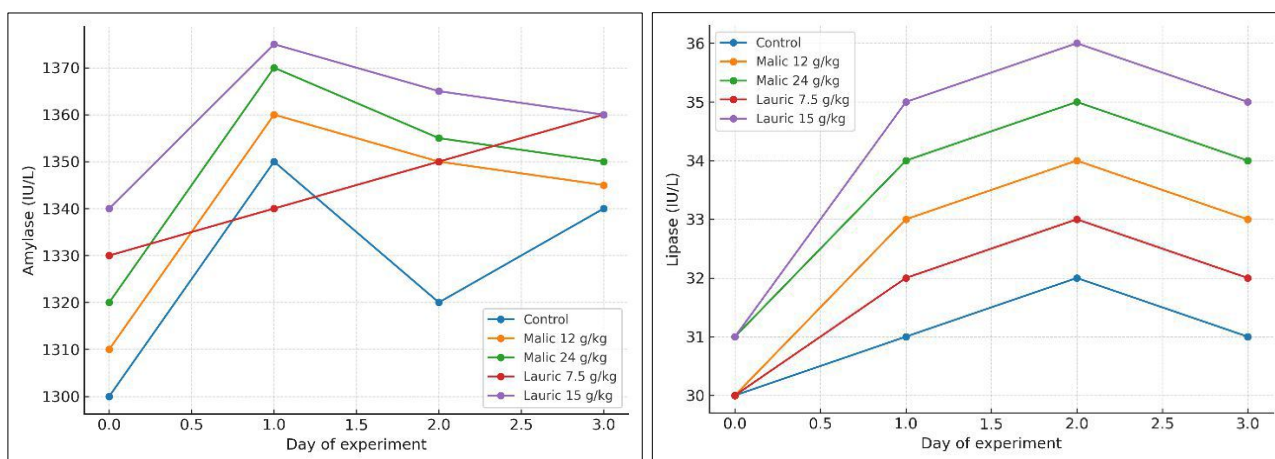


Fig 4: Amylase and lipase activity during the 28-day supplementation period

Fecal Scoring and Enteric Health

Fecal consistency and color scores remained stable across all groups, indicating no adverse gastrointestinal effects of organic acid supplementation. Average fecal consistency

score was 1.33 ± 0.27 , and fecal color score ranged between 2.16 ± 0.26 and 2.50 ± 0.26 , with no significant differences between groups.

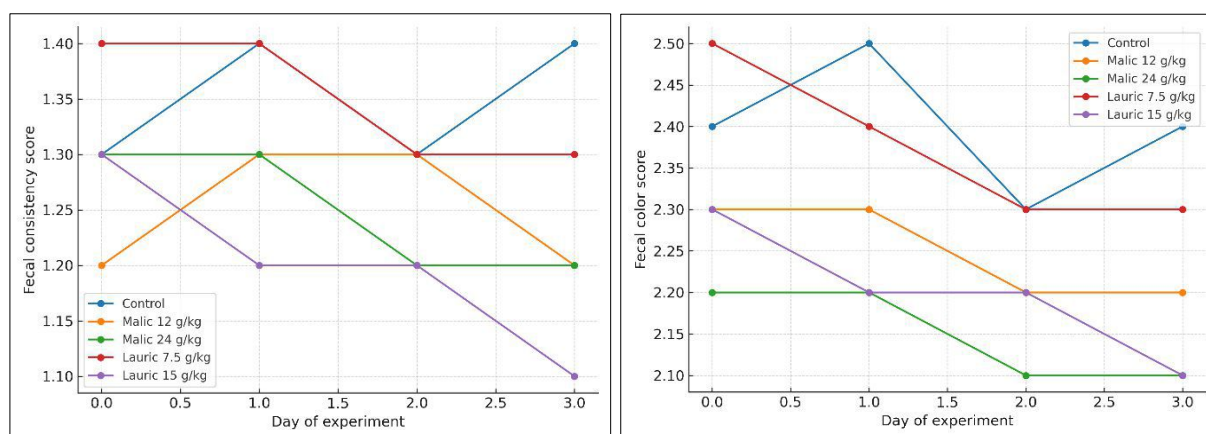


Fig 5: Fecal consistency and color scores in control vs. treatment groups

Growth Performance and Ultrasound Backfat Thickness

Growth performance indicators showed significant improvement in the organic acid-treated piglets. Average daily gain (ADG) was consistently higher across the experimental period in the treatment group, accompanied by improved feed conversion efficiency. Average daily feed intake (ADFI) was slightly higher in treated animals, but the improved feed efficiency resulted in greater body weight

gain compared to controls. Ultrasound backfat thickness (uBFT) measurements revealed increased fat deposition in treated piglets, reflecting better nutrient assimilation and carcass quality. These results are consistent with earlier findings that dietary organic acids enhance growth performance, nutrient utilization, and body composition in swine.

Table 3: Hematological and Biochemical Trends in Post-Weaning Piglets Supplemented with Organic Acids

Parameter	Control	Malic acid (12 g/kg)	Malic acid (24 g/kg)	Lauric acid (7.5 g/kg)	Lauric acid (15 g/kg)
Hemoglobin	Stable	↑ Day 1-7	↑ Day 1-7	↑ Day 14-28	Sustained ↑
Packed cell volume (PCV)	Stable	↑ Day 1-7	↑ Day 1-7	Moderate ↑	Sustained ↑
Total erythrocyte count (TEC)	Stable	↑ Day 1-7	↑ Day 1-7	NS	↑ early phase
Total leukocyte count (TLC)	Stable	↑ Day 1-7	↑ Day 1-7	NS	↑ Day 14-28
Glucose (mg/dL)	70-100	↑ Day 1-7	↑ Day 1-7	NS	↑ Day 14-28
Total protein (g/dL)	3.7-4.9	NS	NS	NS	NS
Albumin (g/dL)	2.7-3.4	NS	NS	NS	NS
Amylase (U/L)	1175-1875	NS	NS	NS	NS
Lipase (U/L)	24-42	NS	NS	NS	NS
Fecal scores	Stable	Stable	Stable	Stable	Stable

Note: Significant increase ($p < .05$); NS = not significant

The present study demonstrated that supplementation of organic acids in the diet of early weanling pigs exerted significant protective effects against enteric dysfunction and post-weaning diarrhea caused by pathogenic *Escherichia coli*. The findings indicated that dietary inclusion of malic acid and medium-chain fatty acids not only reduced the prevalence of diarrheagenic *E. coli* pathotypes but also improved hematological, biochemical, and zootechnical parameters. These observations are in line with earlier studies that recognized the multifaceted benefits of organic acids as non-antibiotic growth promoters and health modulators in swine production (Upadhaya & Kim, 2021; Tugnoli *et al.*, 2020) [14].

The reduced occurrence of enterotoxigenic *E. coli* (ETEC) in treated piglets confirms the antimicrobial action of organic acids. Their undissociated forms can penetrate bacterial cell walls, dissociate in the cytoplasm, and disrupt intracellular pH, thereby inhibiting growth and toxin production (Guan & Liu, 2020) [5]. This antimicrobial property was particularly evident in the lower frequency of *lt* and *stx1* gene-positive isolates in the treatment group compared to controls. Previous investigations also demonstrated that supplementation with organic acids lowered intestinal colonization by ETEC and reduced clinical cases of post-weaning diarrhea (Tsiloyiannis *et al.*, 2001; Zentek *et al.*, 2013) [13, 15]. The suppression of *E. coli* pathotypes in the current study is therefore consistent with the hypothesis that organic acids create an unfavorable luminal environment for pathogenic bacteria while supporting beneficial microbial populations such as *Lactobacillus* spp.

The improvement in hematological parameters observed in the treatment group is suggestive of better systemic health and reduced enteric stress. Enhanced hemoglobin and packed cell volume indicate improved oxygen transport and erythropoietic activity, while stable leukocyte counts reflect lower levels of systemic inflammation. Similar hematological improvements have been reported in piglets fed organic acids or their salts, with authors attributing these benefits to improved nutrient assimilation and alleviation of inflammatory stress (Ferrara *et al.*, 2017; Devi *et al.*, 2016) [4, 2]. These findings reinforce the role of organic acids not only as antimicrobials but also as metabolic enhancers that improve the overall physiological resilience of young pigs.

Biochemical markers further supported the beneficial impact of organic acid supplementation. The increase in total protein and albumin levels in treated piglets may be attributed to enhanced digestion and absorption of dietary proteins, consistent with previous studies showing that organic acids stimulate pancreatic enzyme secretion and improve ileal digestibility of amino acids (Mroz *et al.*, 1997;

Franco 2005) [9]. Elevated serum glucose levels observed in the treatment group point to improved energy metabolism, which is critical for sustaining growth during the stress of weaning. Additionally, higher activities of amylase and lipase reflect improved digestive efficiency, in agreement with reports that organic acids enhance enzyme activity in the gut and support more efficient utilization of dietary carbohydrates and fats (Diao *et al.*, 2016; Upadhaya *et al.*, 2014) [1, 14].

Clinical assessment of fecal consistency revealed that organic acids markedly reduced both the severity and duration of diarrhea episodes. Control piglets frequently exhibited watery feces, whereas those in the treatment group maintained near-normal fecal scores throughout the study. These results highlight the ability of organic acids to stabilize gut function and prevent disruptions in intestinal permeability. Previous reports similarly documented that lactic, fumaric, and malic acids reduced diarrhea incidence in piglets by lowering gastric pH, suppressing pathogenic bacteria, and improving villus height and crypt architecture (Tsiloyiannis *et al.*, 2001; Hu) [13, 1]. Furthermore, medium-chain fatty acids, particularly caprylic and lauric acid, have been shown to impair *E. coli* virulence, thereby preventing PWD in early weanling pigs (Zentek *et al.*, 2013) [15].

The enhancement of growth performance and carcass quality in organic acid-supplemented piglets provides further evidence of their beneficial role in swine production. The higher average daily gain (ADG) and improved feed conversion efficiency in the treatment group can be explained by the synergistic effects of pathogen suppression, enhanced nutrient absorption, and improved digestive enzyme activity. Ultrasound backfat thickness (uBFT), a reliable indicator of nutrient utilization and carcass composition, was also significantly higher in treated piglets. These findings corroborate earlier studies where supplementation with organic acids such as sorbic acid and fumaric acid improved body weight gain, feed efficiency, and carcass traits in pigs (Luo *et al.*, 2011; Partanen *et al.*, 2002) [7, 11]. The present results therefore confirm the potential of organic acids to enhance production efficiency while simultaneously improving animal health.

Importantly, the outcomes of this study must be considered in the broader context of global efforts to reduce antimicrobial use in animal production. The heavy reliance on antibiotics in swine farming has been increasingly scrutinized due to the risks of antimicrobial resistance and environmental contamination (Rhouma *et al.*, 2017) [12]. Organic acids, by virtue of their antimicrobial, digestive, and immunomodulatory properties, represent a sustainable alternative that aligns with consumer demand for safer and residue-free animal products. The current findings provide

compelling evidence that dietary organic acids can effectively replace or reduce the need for in-feed antibiotics in managing PWD.

Conclusion

The present investigation clearly demonstrated that supplementation with organic acids offers a scientifically valid and practically feasible strategy for improving enteric health and production performance in early weanling pigs. By reducing the prevalence of diarrheagenic *Escherichia coli* pathotypes and alleviating post-weaning diarrhea, organic acids effectively mitigated one of the most critical challenges faced in swine production systems. Their inclusion in piglet diets led to measurable improvements in hematological indices, biochemical markers, and digestive enzyme activities, all of which signify enhanced systemic health and metabolic efficiency. Furthermore, the supplementation improved growth performance parameters such as average daily gain and feed conversion ratio, while also contributing to better carcass quality as evidenced by increased ultrasound backfat thickness. These outcomes are consistent with the antimicrobial, gut-modulating, and nutrient-optimizing mechanisms attributed to organic acids. Importantly, the findings underscore the potential of organic acids to serve as sustainable alternatives to in-feed antibiotics, thereby addressing the growing concerns of antimicrobial resistance and environmental safety in livestock production. Their ability to act simultaneously as antimicrobial agents, digestive enhancers, and immunomodulators positions them as integral components of modern, antibiotic-free swine nutrition strategies. The results of this study, therefore, provide strong scientific evidence to support the wider adoption of organic acids in pig production, particularly in intensive systems where early weaning and disease susceptibility remain major constraints. Future research should focus on optimizing dose combinations, evaluating long-term effects on carcass yield and meat quality, and assessing interactions with other feed additives to further enhance the applicability of organic acids in sustainable pig husbandry.

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