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Research article

Influence of Acetic Acid–Based Feed Formulation on Energy and Protein Metabolizability and Growth Performance in Rhode Island Red Chickens (*Gallus gallus domesticus*)

Jess G. Tahanlangit[1*], Julius V. Abela[1], and Milagros C. Bales[2]

[1] College of Agriculture and Food Science, Visayas State University, Philippines
[2] College of Agriculture, Visayas State University, Philippines
* Correspondence: jess.tahanlangit@vsu.edu.ph

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Abstract: The search for alternatives to antibiotic growth promoters in poultry has intensified due to concerns about antimicrobial resistance and food safety. Organic acids, particularly acetic acid, have shown potential in improving gut health, nutrient absorption, and growth performance. This study evaluated the effects of acetic acid–based feed on energy and protein metabolizability, growth performance, and production efficiency in Rhode Island Red chickens (*Gallus gallus domesticus*). Ninety-six one-week-old chicks were randomly assigned to four dietary treatments in a randomized complete block design: T0 (control, 0% AA), T1 (0.3% AA), T2 (1.65% AA), and T3 (3% AA). Home-mixed starter and grower rations were formulated using Brill Feed Formulation Software and fed ad libitum for ten weeks. Growth performance parameters (BW, ADG, WG, FCR, VFI) were measured, while true metabolizable energy (TME) and true metabolizable protein (TMP) were determined using a force-feeding procedure. Data were analyzed using ANOVA and Tukey's HSD ($p<0.05$). No significant differences were observed among treatments for growth performance, TME, or TMP. However, T3 numerically exhibited higher BW, WG, and ADG, and lower FCR. Economically, T3 and T2 had the lowest cost-to-produce (CTP), which was significantly lower than T0 and T1 ($p<0.05$). Although improvements in growth and nutrient utilization were not statistically significant, the consistent numerical trends in T3 suggest a potential benefit of acetic acid inclusion. These results indicate that acetic acid supplementation may enhance economic efficiency without compromising growth performance, supporting its use in sustainable and cost-effective heritage chicken production systems.

Keywords: Acetic acid; metabolizability; growth performance; poultry nutrition; rhode island red

1. Introduction

The poultry industry plays a critical role in global food security, contributing substantially to protein supply and farmer livelihoods. However, one persistent challenge in poultry production is the widespread use of antibiotics as growth promoters and prophylactic agents. While antibiotics at subtherapeutic levels have been shown to improve feed efficiency, growth rate, and disease resistance [1, 2], their long-term use has raised serious concerns regarding antibiotic residues in meat and the development of antimicrobial resistance, posing significant risks to human health [3, 4]. Consequently, many countries have

banned the use of antibiotic growth promoters, prompting a search for safe and effective alternatives. In the Philippines and other ASEAN nations, this restriction has also encouraged the adoption of free-range and heritage chicken production systems, where breeds such as the Rhode Island Red are valued for their adaptability, resilience, and dual-purpose utility [5, 6]. Heritage breeds are often perceived as healthier and better suited to smallholder or "organic" production systems. However, compared to fast-growing commercial broilers, these breeds typically exhibit slower growth rates, reaching market weight within 3–4 months [7, 8]. This slower growth poses economic challenges for farmers, necessitating nutritional strategies that can enhance feed utilization and growth without reliance on synthetic additives. Among promising alternatives, organic acids have attracted considerable attention for their antimicrobial, antifungal, and growth-enhancing properties [9, 10]. Organic acids lower gastrointestinal pH, inhibit pathogenic bacteria, stimulate enzyme activity, and improve nutrient digestibility, thereby enhancing overall growth performance [11, 12]. These compounds—ranging from simple acids such as acetic, formic, and propionic acids to more complex acids such as citric and lactic acids—are also widely used as feed preservatives to reduce microbial contamination [13, 14].

Acetic acid (AA), a short-chain fatty acid produced naturally during fermentation and widely available in commercial form, is of particular interest. Its supplementation in poultry diets has been reported to enhance intestinal morphology, increase villus height and crypt depth, and promote colonization by beneficial microbiota [15, 16]. Furthermore, acetic acid has been shown to improve feed intake, weight gain, and feed conversion ratio [17, 18]. However, findings across studies remain inconsistent. For example, while positive effects were observed at a 0.3% inclusion rate [19], other studies reported neutral or even detrimental outcomes at higher levels, such as 4% [20, 21]. This inconsistency highlights the need for further research to establish optimal inclusion levels and clarify its efficacy across different chicken breeds and production systems. Despite extensive studies on commercial broilers, limited research has been conducted on the application of acetic acid in heritage breeds such as the Rhode Island Red. These chickens, although slower growing, represent an important genetic resource for sustainable poultry farming. Investigating natural feed additives that can improve their nutrient metabolizability and growth performance could offer practical benefits for smallholder farmers and contribute to safer, more sustainable poultry production in the ASEAN region.

Therefore, the present study was conducted to evaluate the effects of acetic acid–based feed formulation on energy and protein metabolizability, as well as growth performance, in Rhode Island Red chickens (*Gallus gallus domesticus*). Specifically, the study aimed to determine the true metabolizable energy (TME) and true metabolizable protein (TMP) of acetic acid–treated diets and to assess their impact on the growth performance of Rhode Island Red chickens under a controlled feeding trial.

2. Materials and Methods

2.1 Procurement of Birds

A total of 96 one-week-old straight-run Rhode Island Red chicks were acquired from a reliable source and were randomly distributed across treatments to ensure a balanced representation of sexes in each group. These birds were fully vaccinated against Newcastle Disease and Gumboro before procurement.

2.2 Preparation of the Study Area

There were twenty-four (24) cages, each with an estimated size of 0.48 sq. m. that can accommodate 4 birds, that is, 0.11 sq. m. or 1.2 sq. ft./bird [22]. The cages were made of coconut lumber and bamboo. The floor height was at least 1.8 meters above the ground to minimize ammonia build- up, while the height from floor to ceiling was at least 2.4 meters for optimum ventilation. A plastic wire mesh was used for the flooring and walls, with hole sizes of 3/4 inch and 1/16 inch, respectively. Polyvinyl chloride (PVC)-based linear feeder was used with a feeding space requirement of 6-7 cm per bird. A ½ gallon round-bubble waterer was placed inside the cages. Before acquiring the experimental animals, the area was cleaned and disinfected.

The experiment was conducted under ambient environmental conditions, with an average temperature of approximately 28-30°C and relative humidity of 70-85%, typical of the local production setting.

2.3 Management of Birds

Upon arrival, the chicks were assigned to a particular cage for brooding and were provided with electrolyte solution to minimize stress from transportation. Papers were used as litter material during the brooding period, and light bulbs as a source of heat. A temperature of 30-33 °C was provided during the first week of age, reduced by 2 °C thereafter, and maintained until the temperature reached 21 °C. A lighting program of 24 hours during the brooding period was implemented, followed by 23 hours of light with 1 hour of darkness per day until market age, using a 5-10-watt bulb [22]. The birds were then weighed after a 2-week adjustment period for the initial record and were assigned to their respective blocks and treatments. The initial body weights (Table 5) of the birds were statistically similar across all treatment groups, indicating uniformity before treatment allocation. A formulated home mixed ration using the Brill Feed Formulation Software of broiler starter (week 1-6), and broiler grower (week 7-10) was given ad libitum (Table 1 and 2). Mortality was monitored daily throughout the experimental period. An average mortality rate of 4% was recorded, with no apparent association with dietary treatments.

Table 1. Home-mixed broiler starter feed ration

	Proportion, %			
Ingredients	T0 (0% AA)	T1 (0.3% AA)	T2 (1.65% AA)	T3 (3% AA)
Yellow corn	42.37	41.77	39.19	37.95
Soybean meal	33.7	33.79	34.22	35.22
Rice bran d1	10	10	10	10
Copra meal	5	5	5	3.40
Coconut oil	3.49	3.7	4.5	5
Molasses	2	2	2	2
MDCP	1.39	1.39	1.39	1.39
Limestone	0.99	0.99	0.99	0.98
Toxin binder	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Meth	0.06	0.06	0.06	0.06
Acetic acid	0	0.3	1.65	3
TOTAL	100	100	100	100

Table 2. Home-mixed broiler grower feed ration

	Proportion, %			
Ingredients	T0 (0% AA)	T1 (0.3% AA)	T2 (1.65% AA)	T3 (3% AA)
Yellow corn	30.78	30.18	30.92	32.13
Soybean meal	24.27	24.36	26.19	28.24
Rice bran d1	19	19	15	15
Copra meal	15	15	15	10.4
Coconut oil	5.69	5.9	5.99	6
Molasses	2	2	2	2
MDCP	1.25	1.25	1.25	1.24
Limestone	0.99	0.99	0.98	0.97
Toxin binder	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Meth	0.02	0.02	0.02	0.02
Acetic acid	0	0.3	1.65	3
TOTAL	100	100	100	100

2.4 Design and Treatments

The study was conducted using a randomized complete block design (RCBD), with cage location within the poultry house serving as the blocking factor to account for potential environmental variation. A total of four dietary treatments were evaluated, each replicated six times with four birds per replicate. In this design, the number of blocks corresponded to the number of replicates. Blocking was implemented to minimize the influence of uncontrolled environmental factors, such as temperature and ventilation gradients within the housing facility. Block effects were included in the statistical model and were found to be not significant ($p>0.05$), indicating that cage location did not substantially influence the measured response variables or treatment comparisons. The birds were randomly assigned to the following treatments: T0 – home mixed ration (control), T1 – home mixed ration (0.3% AA), T2 – home mixed ration (1.65% AA), T3 – home mixed ration (3% AA).

2.5 Preparation of Dietary Treatments

Feed-grade commercial acetic acid (glacial with a concentration of 99%) was purchased at Far Eastern Drug Incorporated, Osmeña Boulevard, Cebu City, Philippines, two weeks before the start of the experiment. The home-mixed rations were formulated according to reference standards [23] using the Brill Feed Formulation Software for broiler nutritional requirements, with locally available ingredients and varying levels of feed-grade acetic acid (Tables 3 and 4). The nutrient composition of the experimental diets was calculated from standard ingredient composition values and was not confirmed by laboratory proximate analysis. Acetic acid was incorporated into the feed by mixing with coconut oil and molasses, ensuring uniform distribution throughout the ration before feeding. The stability and retention of acetic acid in the formulated diets were not directly measured during storage. However, diets were prepared in small batches and provided daily to minimize potential volatilization losses, given acetic acid's volatility. Water intake was not quantitatively measured in this study; however, clean drinking water was provided ad libitum throughout the experimental period.

Table 3. Nutrient composition of home-mixed starter feed ration with AA

Chemical Composition	Starter Feeds			
	T0 (0%)	T1 (0.3%)	T2 (1.65%)	T3 (3%)
Dry matter (%)	87.05	86.80	85.71	84.50
Moisture (%)	12.95	13.2	14.29	15.5
Metabolizable Energy (kcal/kg)	2,698.1750	2,699.6250	2,700.5500	2,699.9570
Crude Protein	19.30	19.30	19.30	19.30
Crude Fat	7.21	7.40	8.12	8.43
Crude Ash	3.80	3.79	3.78	3.73

Table 4. Nutrient composition of home-mixed grower feed ration with AA

Chemical Composition	Grower Feeds			
	T0 (0%)	T1 (0.3%)	T2 (1.65%)	T3 (3%)
Dry matter (%)	89.80	90.42	88.85	88.03
Moisture (%)	10.20	9.58	11.15	11.97
Metabolizable Energy (kcal/kg)	2,698.9800	2,700.4300	2,700.0170	2,699.4930
Crude Protein	18	17.99	17.99	18
Crude Fat	10.93	11.11	10.86	10.48
Crude Ash	4.25	4.24	4.12	3.97

Due to the inclusion of varying levels of acetic acid, minor adjustments to other feed ingredients were necessary to maintain a total formulation of 100%. Consequently, slight variations in nutrient composition, particularly crude fat, were observed across treatments. Although efforts were made to maintain comparable nutritional profiles, the diets were not strictly iso-energetic or iso-nitrogenous. This approach was adopted to reflect practical feed formulation conditions commonly encountered in smallholder production systems.

2.6 Digestibility Trial

The determination of true metabolizable energy (TME) and true metabolizable protein (TMP) was conducted following the force-feeding method [24]. A total of twenty (20) birds (five per treatment) were randomly selected and individually housed in metabolism cages. Each bird was fasted for 24 hours before force-feeding to ensure gastrointestinal emptying. Subsequently, each bird was crop-intubated with 20 g of the respective grower diet on an as-fed basis. A plastic funnel (5.5 mm internal diameter) was placed in the crop via the esophagus, and force-feeding was performed. The feed was inserted into the tube as mash (4.76 mm in diameter) and pushed into the crop with a wooden rod. Fusing a funnel to the top of the funnel aided in mash entrance. The birds were returned to their cages following feeding. Under each cage, a plastic dish was placed to collect excreta. The birds were weighed again exactly 24 hours after force-feeding, and their voided excreta were quantitatively collected. The excreta were air-dried, weighed, oven-dried, weighed again, and pulverized to pass through a 20-mesh screen for further analysis.

For the estimation of endogenous energy and nitrogen losses, an additional four (4) birds were fasted for 48 hours without feed intake, and excreta were collected over the same period. The endogenous loss values obtained from these birds were pooled and used as correction factors in the calculation of TME and TMP for all treatments. The gross energy and crude protein composition of ground feed and excreta were determined, and TME and TMP were computed, respectively. All birds were acclimatized to handling and individual cages before the trial to minimize stress during force-feeding and excreta collection. The following equations were used for the calculation of true metabolizable energy (TME) and true metabolizable protein (TMP):

1. True Metabolizable Energy (TME %):

$$\text{TME (\%)} = \frac{\text{GE}feed \; - \; (\text{GE}excreta \; - \; \text{GE}endogenous)}{\text{Feed Intake}} \text{ X } 100$$

2. True Metabolizable Protein (TMP %):

$$\text{TMP (\%)} = \frac{\text{P}feed \; - \; (\text{P}excreta \; - \; \text{P}endogenous)}{\text{Feed Intake}} \text{ X } 100$$

where GE represents gross energy and P represents nitrogen content.

2.7 Statistical Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA) under a randomized complete block design (RCBD) using the PROC GLM procedure of SAS version 9. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test, and differences were considered statistically significant at $p < 0.05$. Results are presented as mean ± standard error of the mean (SEM). The Tukey HSD test was employed to control for Type I error across multiple pairwise comparisons among treatments.

3. Results and Discussion

3.1 Growth Performance

Table 5 shows the growth performance of the Rhode Island Red chickens fed with different acetic acid-based formulations according to voluntary feed intake (VFI), body weight (BW), average daily gain (ADG), weight gain (WG), and feed conversion ratio (FCR). Based on the result, there were no significant differences in terms of growth performance across all parameters ($P>0.05$), however, T3 (3% AA) exhibited numerically improved values compared to other treatments in terms of BW, ADG, WG, and with lower FCR, with 900.45 grams, 10.01 grams, 715.28 grams, and 3.62, respectively. The initial body weight (IBW) of birds was statistically comparable across treatments ($p > 0.05$), indicating homogeneity among experimental groups before dietary treatment.

The absence of significant differences in VFI indicates that dietary inclusion of AA did not adversely affect feed palatability under the conditions of this study. This finding is consistent with previous reports [15, 19], who likewise observed no significant changes in feed intake with organic acid supplementation. However, other studies have reported decreased feed intake at higher inclusion levels, which was attributed to reduced palatability due to the strong acidic taste of organic acids [21, 25, 26]. These contrasting findings suggest that the response of feed intake

to organic acid supplementation may vary depending on acid concentration, method of administration (feed vs. water), and the adaptability of the birds.

Table 5. Growth performance of Rhode Island Red chickens fed an acetic-acid-based formulation

Parameters	T0 (control)	T1 (0.3%)	T2 (1.65%)	T3 (3%)	SEM	p-value	DF (treatment, error)
IBW (g)	186.25	186.67	183.71	185.17	10.85	0.9974	3, 15
VFI (g)	2823.96	2541.84	2542.64	2589.31	141.02	0.9327	
BW (g)	850.71	812.74	863.56	900.45	66.04	0.8247	
ADG (g)	9.49	8.84	9.71	10.01	0.83	0.7854	
WG (g)	664.46	626.07	679.85	715.28	61.94	0.7854	
FCR	4.25	4.06	3.74	3.62	0.3686	0.6143	

Although BW, ADG, and WG were not significantly affected, the observed numerical increases at higher AA inclusion levels indicate a trend toward improved growth performance. Similar improvements have been reported in broilers supplemented with organic acids, where enhanced growth was associated with improved nutrient digestibility and utilization [15, 19]. The potential mechanisms underlying these effects include the ability of organic acids to lower gastrointestinal pH, thereby creating a favorable environment for digestive enzyme activity and inhibiting the proliferation of pathogenic microorganisms. This, in turn, enhances nutrient absorption and utilization, which may explain the improved growth trends observed in birds receiving higher levels of AA. Furthermore, considering that Rhode Island Red chickens are relatively slow-growing compared to commercial broilers [7, 8], the numerical improvements observed in this study suggest that dietary acidification may help enhance their growth potential under appropriate management conditions.

The trend toward improved FCR with increasing AA inclusion further supports the role of organic acids in enhancing feed efficiency. Improved FCR is often associated with better intestinal morphology, including increased villus height and surface area, which facilitates more efficient nutrient absorption. Previous studies have demonstrated that organic acid supplementation can improve intestinal structure and function, thereby enhancing feed utilization [15, 19]. In addition, the antimicrobial properties of acetic acid may reduce the load of harmful bacteria in the gastrointestinal tract, improving gut health and reducing competition for nutrients. Enhanced FCR has also been reported in broilers supplemented with organic acids under both normal and disease-challenged conditions [16, 17, 18, 27], although some studies have reported inconsistent responses depending on inclusion level [26].

While the beneficial effects of organic acids are often attributed to their ability to reduce gastrointestinal pH and modulate microbial populations, intestinal pH was not measured in this study. Thus, the proposed mechanism remains inferential and should be interpreted with caution. It should also be noted that the observed effects cannot be attributed solely to acetic acid supplementation, as incremental inclusion levels required adjustments in other dietary components, resulting in slight variations in fat content among treatments. These compositional differences may have contributed to the numerical improvements observed in growth performance parameters. Therefore, the results should be interpreted as the combined effect of acetic acid inclusion and associated dietary modifications rather than the isolated impact of acetic acid alone. The absence of statistically significant differences across treatments may be partly attributed to sample size limitations and inherent variability in biological responses. Although the number of replicates used in this study is consistent with similar poultry nutrition experiments, future studies with larger sample sizes may provide greater statistical power to detect subtle treatment effects.

3.2. True Metabolizable Energy (TME) and True Metabolizable Protein (TMP) of Grower Feeds Treated with Acetic Acid

Table 6 presents the gross energy (GE) and crude protein (CP) content of chicken excreta with acetic acid (AA) supplementation. The GE values for the force-fed chickens were 3,588.80 kcal for T0 (control group), 3,391 kcal for T1 (0.3% AA), 3,318.30 kcal for T2 (1.65% AA), and 3,362.30 kcal for T3 (3% AA). In contrast, the GE values of the fasted chickens were 2,083 kcal, 1,750 kcal, 1,660 kcal, and 1,507 kcal for T0, T1, T2, and T3 groups, respectively. Regarding crude protein (CP) percent, the excreta of force-fed chickens contained 19.17%

in T0, 18.12% in T1, 18.82% in T2, and 19.58% in T3. In fasted chickens, the CP content was 6.20%, 5.97%, 5.36%, and 5.19% in the T0, T1, T2, and T3 groups, respectively.

Table 6. Gross Energy (GE) and Crude Protein (CP) of Excreta from Force-Fed and Fasted Rhode Island Red Chickens

Chemical Composition	T0 (0%)	T1 (0.3%)	T2 (1.65%)	T3 (3%)
		Forced-Fed Chicken Excreta		
Gross Energy (kcal)	3588.80	3391	3318.30	3362.30
Crude Protein (%)	19.17	18.12	18.82	19.58
		Fasted Chicken Excreta		
Gross Energy (kcal)	2083	1750	1660	1507
Crude Protein (%)	6.2	5.97	5.36	5.19

As shown in Table 7, there were no statistically significant differences ($p>0.05$) in true metabolizable energy (TME) or true metabolizable protein (TMP) among treatments. Nevertheless, numerically higher values were observed in T3 (3% AA), which recorded 70.99% TME and 61.93% TMP, suggesting improved dietary energy and protein absorption when acetic acid was included in the feed. Similarly, TME and TMP values were also comparatively higher in T2 (1.65% AA; 70.50% TME and 61.24% TMP) and T1 (0.3% AA; 69.82% TME and 60.47% TMP) relative to the control group, which recorded 68.63% TME and 60.26% TMP.

Table 7. Percent True Metabolizable Energy (TME) and True Metabolizable Protein (TMP) of Grower Feeds with the Inclusion of Acetic Acid

Parameters	Grower Feeds				SEM	p-value	DF (treatment, error)
	T0 (control)	T1 (0.3%)	T2 (1.65%)	T3 (3%)			
TME (%)	68.63	69.82	70.5	70.99	2.7556	0.9347	3, 12
TMP (%)	60.26	60.47	61.24	61.93	0.8838	0.5432	

In the poultry industry, good intestinal health is critical for achieving target growth rates and feed efficiency, i.e., improved nutrient absorption. Studies regarding the use of organic acids significantly increased the villus width, height, and area of the duodenum, jejunum, and ileum. Studies have shown that dietary organic acids can improve intestinal morphology in broilers. Broilers fed diets supplemented with formic acid exhibited longer intestinal villi compared to the control group [28], while similar improvements were observed in broilers supplemented with butyrate [29,30]. Significant ($P<0.05$) increases in the villus heights of the duodenum, jejunum, and ileum were also observed following AA supplementation [19]. Moreover, using organic acids such as formic acid, citric acid, and AA improved the nutrient digestibility of energy, protein, ether extract, crude fiber, and nitrogen-free extract in broilers. Supplementation with formic acid [28] and citric acid [31] also improved the ileal digestibility of DM and CP in a broiler grower diet. The ability of organic acids, such as AA, to improve intestinal health, morphology, and overall nutrient absorption capacity could explain why chickens fed the treated feeds showed better TME and TMP. The absence of statistically significant differences in TME and TMP across treatments may be partly due to the relatively small sample size in the digestibility trial. With only five replicates per treatment and limited birds for estimating endogenous loss, the statistical power to detect treatment effects was reduced. Nevertheless, consistent numerical trends across treatments suggest potential biological relevance and warrant further investigation with larger sample sizes.

3.3 Cost to Produce (CTP) and Chicken Production Efficiency Factor (CPEF)

The calculated Cost to Produce (CTP) and Chicken Production Efficiency Factor (CPEF) of Rhode Island Red chickens fed with Acetic-acid-based feed formulation are presented in Table 6. The CTP was determined by dividing the total cost of consumables, including chick, feed, and medication expenses (Table 9), by the final body weight (BW) of the chickens. In contrast, the CPEF serves as a comprehensive indicator of overall chicken

performance and was calculated by dividing the BW by the feed conversion ratio (FCR) and multiplying the result by 100. Acetic acid–based feed formulation significantly affected the cost to produce (CTP) live weight ($p < 0.0001$). The lowest CTP values were observed at 3% (T_3) and 1.65% (T_2) inclusion, which were 2.8% and 2.3% lower, respectively, than the control. These results indicate that higher inclusion rates improve economic efficiency, with T_3 providing the greatest cost reduction. The slight numerical advantage of T_3 over T_2 suggests that further improvements may still occur beyond 1.65%, although the effect appears to approach a plateau. The significant reduction in cost-to-produce (CTP) observed at higher acetic acid inclusion levels was primarily driven by differences in feed formulation costs rather than improvements in growth performance alone. Variations in ingredient composition, particularly the inclusion of locally available feed components and adjustments in energy sources, contributed to lower feed costs in T_2 and T_3 treatments. In contrast, 0.3% inclusion (T_1) yielded a slightly higher CTP than the control, indicating that suboptimal dosing does not confer economic benefit. Numerical increases in Chicken Production Efficiency Factor (CPEF) were also noted for T_3 and T_2 compared to control, but differences were not statistically significant ($p = 0.2778$). Overall, these findings suggest that including 3% acetic acid offers the greatest cost advantage without negatively affecting production efficiency, making it a promising strategy to improve profitability in Rhode Island Red chicken production.

Table 8. CTP and CPEF of Rhode Island Red chickens fed acetic-acid based formulation

Parameter	T0 (Control)	T1 (0.3%)	T2 (1.65%)	T3 (3%)	SEM	p-value	DF (treatment, error)
CTP	214.21[b]	215.62[a]	209.17[c]	208.32[c]	0.26	<.0001	3, 15
CPEF	21.88	20.75	24.70	25.63	1.94	0.2778	

* Means within a column with different superscripts (a–c) are significantly different at $p<0.05$ based on Tukey's HSD test. Superscripts do not indicate magnitude ranking but statistical grouping.

Table 9. Total Production Cost Per Treatment

Costs/bird	T0 (Control)	T1 (0.3%)	T2 (1.65%)	T3 (3%)
Chick Cost	100	100	100	100
Feed Cost	79.83	73.05	78.29	85.03
Medication (3% of Feed Cost)	2.40	2.19	2.35	2.55
Total	182.23	175.24	180.64	187.58

4. Conclusions

The inclusion of acetic acid in home-mixed rations for Rhode Island Red chickens resulted in numerical improvements in nutrient utilization, growth performance, and economic efficiency. Although differences in growth parameters and metabolizability were not statistically significant ($p > 0.05$), a consistent trend toward improved performance was observed with increasing levels of acetic acid, particularly at 3% inclusion. Birds receiving the 3% treatment exhibited higher body weight and weight gain and a lower (more favorable) feed conversion ratio than those in other treatments. In addition, cost-to-produce values differed significantly among treatments ($p < 0.05$), with the 1.65% and 3% acetic acid groups among the lowest, suggesting potential economic advantages. However, these findings should be interpreted with caution, as the observed effects may not be solely attributable to acetic acid itself but could also be influenced by concurrent changes in dietary nutrient composition. Furthermore, the nutrient composition of the experimental diets was based on calculated values rather than laboratory analysis, which may introduce minor discrepancies between expected and actual nutrient content. While the consistent numerical trends observed may suggest potential biological relevance, further validation is required. Future studies should employ larger sample sizes, incorporate proximate analysis of experimental diets, and use strictly isoenergetic and iso-nitrogenous formulations to isolate the effects of acetic acid supplementation better. A longer feeding trial up to market age (16–18 weeks) may also be considered to validate sustained effects on body weight and carcass traits, as well as to conduct histological and

microbiological analyses better to understand its impact on gut health and microbial balance. Additionally, economic feasibility studies in both smallholder and commercial systems are needed to confirm cost–benefit outcomes, while comparative trials with other organic acids or acidifier blends may help determine potential synergistic effects on growth and nutrient digestibility.

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