Research Article

Effects of *Psidium guajava*, *Cymbopogon citratus*, and *Allium sativum* Wine Infusion on Growth Performance, Survival Rate, and Fecal Coccidial Oocyst Shedding in Noi Crossbred Chickens

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Abstract | Coccidial infection remains an important enteric problem in chicken production, particularly in chickens reared on litter floors under farm conditions. Plant-based supplements may offer a practical supportive approach for improving growth performance and reducing detectable fecal coccidial oocyst shedding. This study evaluated the effects of *Psidium guajava*, *Cymbopogon citratus*, and *Allium sativum* wine infusion (PCAW) on growth performance, feed conversion ratio, survival rate, and fecal coccidial oocyst shedding in Noi crossbred chickens. Noi crossbred chickens were assigned to four treatments, including a control group without PCAW and three PCAW-supplemented groups receiving 0.5%, 0.6%, or 0.7% PCAW through drinking water. Body weight, body weight gain, feed intake, feed conversion ratio, survival rate, and fecal coccidial oocyst shedding were evaluated during the 70-day experimental period. PCAW supplementation significantly affected final body weight, body weight gain, and feed conversion ratio ($P<0.001$), whereas feed intake did not differ among treatments ($P=0.981$). The PCAW 0.6% group showed the highest final body weight (998.67 g/chicken), the highest body weight gain (937.37 g/chicken), and the lowest feed conversion ratio (3.01). PCAW-supplemented groups also showed lower detectable fecal coccidial oocyst shedding than the control group, with the most consistent response observed at 0.6%. Survival reached 100.00% in all PCAW-supplemented groups and 95.00% in the control group, but this difference was not statistically significant ($P=0.49$) and should be interpreted only as a numerical observation rather than a proven survival benefit. Under the conditions of this 70-day farm trial, PCAW at 0.6% showed the most favorable response in improving growth performance and feed efficiency while reducing detectable fecal coccidial oocyst shedding in Noi crossbred chickens. However, PCAW should not be interpreted as a confirmed preventive or therapeutic treatment for coccidiosis. Further studies with standardized PCAW characterization, residual alcohol assessment, McMaster OPG quantification, and *Eimeria* species identification are needed before broader farm-level recommendations can be made.

Keywords | Coccidial oocyst shedding, Feed conversion ratio, Herbal wine infusion, Noi crossbred chicken, PCAW

Received | May 06, 2026; **Accepted** | June 18, 2026; **Published** | July 19, 2026
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Citation | Tam QTT, Vinh TP, Bich VTN, Nhan P (2026). Effects of *Psidium guajava*, *Cymbopogon citratus*, and *Allium sativum* wine infusion on growth performance, survival rate, and fecal coccidial oocyst shedding in Noi crossbred chickens. Adv. Anim. Vet. Sci., 14(7):1538-1552.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.7.1538.1552
ISSN (Online) | 2307-8316

INTRODUCTION

Poultry production plays a critical role in food security and rural livelihoods, particularly in developing countries (Chuc *et al*., 2026). In Vietnam, chicken production is an important source of animal protein and income for rural households. However, intestinal diseases continue to limit productivity, especially in young chickens raised under

farm conditions. Among these diseases, coccidial infection is one of the most important parasitic problems in poultry production, leading to reduced growth, poor feed efficiency, intestinal damage, and economic losses (Blake *et al*., 2020; El-Shall *et al*., 2024). In practice, subclinical coccidial infection can persist in floor-reared poultry systems with litter bedding, where oocysts may accumulate and remain infectious in the environment despite routine control measures (Chuc and Nhan, 2026).

The control of coccidiosis remains difficult because *Eimeria* oocysts can survive in poultry houses and maintain infection pressure under farm conditions. In addition, concerns about the long-term use of conventional anticoccidial agents, including ionophores and chemical drugs, have encouraged interest in supportive strategies that may reduce dependence on chemotherapeutic products in poultry production. Bortoluzzi *et al*. (2025) noted that reduced efficacy of anticoccidial agents due to resistance remains an important concern in coccidiosis control. Peek and Landman (2011) also suggested that the increasing occurrence of drug resistance has created a need to develop drug-free products and reduce dependence on chemotherapeutic agents in animal production. In the present study, drug resistance was considered only as a general background concern in coccidiosis control, not as a confirmed condition on the experimental farm. Therefore, this study did not aim to test PCAW as a replacement for anticoccidial drugs, but rather as a practical plant-based supportive supplement under farm conditions. Plant-derived feed additives have received increasing attention as sustainable alternatives or supportive tools in poultry diets (Habibi and Ghahtan, 2019; Sugiharto, 2016).

Guava leaves (*Psidium guajava*), lemongrass (*Cymbopogon citratus*), and garlic (*Allium sativum*) are locally available medicinal plants with recognized biological properties. Guava is a tropical plant widely distributed in tropical America and Southeast Asia. In traditional medicine, different parts of the plant, particularly the leaves, have been used to manage gastrointestinal disorders and parasitic infections in these regions (Khadhri *et al*., 2014). Previous studies have also reported several biological properties of guava and its essential oil, including antimicrobial and antiparasitic activities (de Souza *et al*., 2017; Machado *et al*., 2018). Lemongrass is widely cultivated in tropical and subtropical regions (Li et al., 2020). Its essential oil, commonly known as lemongrass oil, contains citral as the major bioactive component (Saddiq and Khayyat, 2010). Previous studies have reported that lemongrass oil exhibits several pharmacological properties, including antiparasitic, antioxidant, antimicrobial, and anti-inflammatory activities (Cheel *et al*., 2005; Saddiq and Khayyat, 2010; Costa *et al*., 2016; Méabed *et al*., 2018). Garlic has long been used as both a culinary ingredient and a medicinal plant (Eftekhari and Ghaniei, 2023). It belongs to the genus *Allium*, together with onion, leek, and chive (Sharifi-Rad *et al*., 2016). The characteristic aroma and flavor of garlic are largely associated with sulfur-containing compounds, particularly allicin (Bastaki *et al*., 2021; Lengbiye *et al*., 2020). Studies indicate that allicin can inhibit the growth and development of *Eimeria* parasites by disrupting parasite cell membrane integrity and interfering with their replication process (Abd-Elrahman *et al*., 2022; El-Khtam *et al*., 2014). In addition, allicin may stimulate host immune responses, thereby supporting the ability of chickens to resist parasitic infections (Arreola *et al*., 2015).

Although guava, lemongrass, and garlic have been investigated in poultry and parasitic control studies, most previous work has focused on single plant materials, dried powders, essential oils, aqueous extracts, ethanol extracts, or chemically defined preparations. These forms may differ in extraction efficiency, bioactive compound profile, dosage standardization, and practical applicability under farm conditions. In local and smallholder production systems, herbal wine infusion is a traditional and easily prepared form that uses an ethanol-water solvent to extract both water-soluble and moderately lipophilic compounds from plant materials. However, information remains limited on the practical use of a combined herbal wine infusion administered through drinking water, especially in Noi crossbred chickens raised under farm conditions. The specific gap addressed in this study is therefore the evaluation of a locally prepared ethanol-water herbal infusion combining *Psidium guajava* leaves, *Cymbopogon citratus*, and *Allium sativum* as a farm-level supportive supplement. The novelty lies in the preparation form, the combined plant formulation, the drinking-water administration route, the Noi crossbred chicken type, and the evaluation under natural farm exposure rather than a controlled coccidiosis challenge model.

In this study, PCAW was evaluated as a whole locally prepared herbal wine infusion, not as a chemically standardized extract or as isolated plant bioactive compounds. Because the chemical composition of PCAW, residual alcohol concentration in drinking water, and an alcohol-only control treatment were not included, the observed responses cannot be attributed exclusively to the herbal components. Instead, they should be interpreted as the response to the complete PCAW preparation under the present farm conditions. Future studies should include phytochemical characterization, residual alcohol assessment, and an alcohol-only control to separate the effects of the ethanol-water solvent from those of the plant materials.

Noi chicken is a native Vietnamese chicken breed valued for its adaptability to local environmental, nutritional, and management conditions. Noi crossbred chickens are commonly raised under smallholder and farm conditions in Vietnam because they retain desirable traits of the native genetic background, such as adaptability, meat quality, and natural robustness, while offering improved productive performance. However, information on the use of locally prepared herbal wine infusions to support growth performance and reduce detectable fecal coccidial oocyst shedding in this chicken type remains limited. Therefore, this study was conducted to evaluate the effects of PCAW, a wine infusion prepared from *Psidium guajava* leaves, *Cymbopogon citratus*, and *Allium sativum*, on body weight, feed conversion ratio, survival rate, and detectable fecal coccidial oocyst shedding in Noi crossbred chickens.

MATERIALS AND METHODS

Study animals

The experiment was conducted at a chicken farm in Long Ho Commune, Vinh Long Province, Vietnam. Sample processing and microscopic examination were performed at the laboratory of the Department of Veterinary Medicine, Vinh Long University of Technology Education.

A total of 160 one-day-old Noi crossbred chicks were used in this study. The chicks had an average initial body weight of approximately 32 g and were clinically healthy at the beginning of the experiment. Before random allocation, chicks were selected for uniformity in age, clinical health status, and body weight to reduce initial variation among treatments. The chicks were randomly allocated to four treatment groups, with four replicates per treatment and 10 chicks per replicate. Each replicate pen contained five male and five female chicks to maintain the same sex ratio across treatments.

Sex-specific growth analysis was not performed because the study was designed to evaluate the overall treatment effect of PCAW. In addition, feed intake and feed conversion ratio were recorded at the replicate pen level and could not be separated accurately for male and female chickens. Therefore, sex was controlled through balanced allocation rather than analyzed as a separate experimental factor.

All experimental chickens were reared under the same housing, feeding, and management conditions throughout the experimental period. The chickens were kept in physically separated floor pens with litter bedding to reduce cross-contact among treatment groups. Feed and drinking water were provided *ad libitum*. Vaccination and routine health management were performed according to the recommendations of the Department of Animal Husbandry, Veterinary Medicine and Fisheries of Vinh Long Province.

Preparation of *Psidium guajava* leaves, *Cymbopogon citratus*, and *Allium sativum* wine

The *Psidium guajava* leaves, *Cymbopogon citratus*, and *Allium sativum* wine infusion (PCAW) was prepared as a locally practical herbal wine infusion using fresh guava leaves, fresh lemongrass, and fresh garlic. Fresh garlic bulbs were peeled and thinly sliced. Young guava leaves and lemongrass were washed, drained, and cut into small pieces before infusion.

The infusion was prepared using 1 kg of fresh garlic, 50 g of young guava leaves, and 50 g of lemongrass in 2 L of 40% rice wine. The 40% wine used for infusion was commercial rice wine purchased from a local supplier in Vinh Long Province, Vietnam, and the same batch of wine was used for PCAW preparation during the study. The ingredient ratio corresponded to 500 g garlic, 25 g guava leaves, and 25 g lemongrass per 1 L of wine. The formulation was garlic-dominant because garlic was used as the main herbal component in this local practical preparation, while guava leaves and lemongrass were included at lower levels as supporting plant materials. The lower amounts of guava leaves and lemongrass were selected to reduce excessive bitterness, strong aroma, and possible palatability problems when PCAW was diluted in drinking water. This ratio was used as a practical farm-level formulation rather than as a chemically optimized extraction ratio.

All plant materials were placed into a clean glass container, and 40% rice wine was added until the materials were fully submerged. The container was tightly closed and kept in a cool, dry place at room temperature away from direct sunlight for 15 days. The 15-day infusion period was selected based on local practical preparation of herbal wine infusion, allowing sufficient contact time between the plant materials and the ethanol-water solvent before use. However, no chemical extraction kinetics or optimization test was performed to confirm that this period produced maximal concentrations of bioactive compounds.

After the 15-day infusion period, the liquid portion was filtered through clean gauze, transferred into clean glass bottles, tightly capped, and stored in a cool, dry place away from direct sunlight until use. The concentrations of allicin, citral, tannins, total phenolics, and other bioactive compounds in the final PCAW were not measured. Chemical stability of PCAW during storage was also not evaluated. Therefore, PCAW in this study should be considered a locally prepared herbal wine infusion rather than a chemically standardized product. To improve reproducibility, the plant material amounts,

wine concentration, plant-to-wine ratio, infusion duration, storage condition, and dilution levels in drinking water were described in detail.

Feed and feeding

All experimental chickens were provided with the same commercial complete feed formulated for growing chickens throughout the experimental period. The basal diet contained a declared metabolizable energy level of approximately 3,000 kcal/kg and a minimum crude protein level of 20%. The feed was formulated using common ingredients, including maize, broken rice, fish meal, soybean meal, rice bran, and other minor ingredients. The declared calcium and phosphorus levels were approximately 0.8 to 1.2% and 0.5 to 1.0%, respectively. The same basal diet was used for all treatment groups to minimize nutritional variation among treatments. Feed was offered *ad libitum*, and fresh drinking water was continuously available.

The nutrient values reported for the basal diet were based on the commercial feed label and declared nutrient specification rather than laboratory proximate analysis. Feed samples were not chemically analyzed for crude protein, crude fat, crude fibre, ash, calcium, or phosphorus in the present study. Therefore, the diet composition should be interpreted as declared nutrient values rather than laboratory-confirmed values.

PCAW was supplied through drinking water according to the assigned treatment levels. The control group received drinking water without PCAW, whereas the supplemented groups received PCAW at concentrations of 0.5%, 0.6%, or 0.7%, corresponding to 50, 60, or 70 mL PCAW per 10 L of drinking water, respectively. PCAW-supplemented drinking water was freshly prepared at each administration and was provided once every two days from 1 day of age until the end of the 70-day experiment. On non-PCAW days, chickens received clean drinking water without PCAW. PCAW-supplemented drinking water was not kept in the drinkers for 48 hours.

The every-other-day schedule was selected as a practical farm-level supplementation approach to avoid continuous exposure of young chickens to the herbal wine infusion while still allowing repeated supplementation during the experimental period. Daily administration was not tested, and the pharmacokinetics or persistence of PCAW bioactive compounds in chickens were not evaluated. Therefore, this schedule should be interpreted as a practical management approach rather than an optimized dosing regimen.

Feed offered and feed refusals were recorded at the replicate pen level to determine feed intake. Feed conversion ratio was calculated based on total feed intake and body weight gain during the experimental period. Water intake was not measured in this study. Therefore, the actual PCAW intake per chicken could not be calculated, and possible differences in drinking behavior among treatments cannot be excluded. The PCAW levels reported in this study refer to the dilution concentrations prepared in drinking water, not to the confirmed amount consumed by each chicken.

Experimental design

The experiment was arranged in a completely randomized design with four treatments and four replicates per treatment, as shown in Table 1. A total of 160 one-day-old Noi crossbred chicks were randomly assigned to 16 experimental pens, with 10 chicks per pen. Each replicate pen contained five male and five female chicks to maintain the same sex ratio across treatments.

The replicate pens were physically separated within the farm, and each pen had its own feeder and drinker. Drinking water was prepared and provided separately for each treatment group according to the assigned PCAW concentration. Control pens received only clean drinking water without PCAW. This arrangement was used to avoid accidental sharing of drinking water and to reduce the risk of cross-contamination among treatment groups.

Table 1: Experimental arrangement.

Replicate	Treatment 1 (control)	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%
1	10 chickens	10 chickens	10 chickens	10 chickens
2	10 chickens	10 chickens	10 chickens	10 chickens
3	10 chickens	10 chickens	10 chickens	10 chickens
4	10 chickens	10 chickens	10 chickens	10 chickens
Total	40 chickens	40 chickens	40 chickens	40 chickens

PCAW = *Psidium guajava*, *Cymbopogon citratus*, and *Allium sativum* wine infusion. **Table 1** presents only the experimental layout; therefore, standard deviation and statistical values are not applicable.

The treatments were based on different concentrations of PCAW in drinking water. PCAW refers to *Psidium guajava*, *Cymbopogon citratus*, and *Allium sativum* wine infusion, prepared from guava leaves, lemongrass, and garlic. The experimental treatments were as follows: Treatment 1 was the control group receiving the basal diet and drinking water without PCAW; Treatment 2 received the basal diet and drinking water supplemented with 0.5% PCAW; Treatment 3 received the basal diet and drinking water supplemented with 0.6% PCAW; and Treatment 4 received the basal diet and drinking water supplemented with 0.7% PCAW. PCAW was administered through drinking water once every two days from 1 day of age

until the end of the 70-day experimental period. All experimental chickens were managed under the same housing, feeding, and health-care conditions throughout the study.

Detection of coccidial oocysts by the flotation method

Coccidial oocysts in fecal samples were detected using Willis' flotation method. This method is based on the principle that saturated sodium chloride solution has a higher specific gravity than coccidial oocysts, allowing the oocysts to float to the surface of the solution and adhere to the coverslip for microscopic examination.

Fresh fecal samples were collected once weekly in the early morning from the experimental chickens throughout the study period. Sampling was conducted at 8 to 14, 15 to 21, 22 to 28, 29 to 35, 36 to 42, 43 to 49, 50 to 56, 57 to 63, and 64 to 70 days of age. Only freshly excreted feces were collected to minimize contamination from the litter. Each fecal sample, weighing approximately 5 g, was placed in an individual zipper bag and clearly labeled with the sample number, treatment, replicate, breed, age, and date of collection. The samples were kept in an ice box during transport to the laboratory and examined for coccidial oocysts using Willis' flotation method. All samples were analyzed within 2 to 3 days after collection. During storage, the samples were kept refrigerated at 5 to 10°C.

The saturated sodium chloride solution was prepared by gradually adding NaCl to distilled water while stirring continuously until no more salt dissolved, using approximately 360 g NaCl per litre of water. The solution was then filtered through filter paper to remove undissolved particles and obtain a clear saturated salt solution.

For fecal examination, approximately 2 to 3 g of feces were placed into a clean glass vial. Saturated NaCl solution was added to one third of the vial, and the feces were thoroughly mixed using a glass rod. Additional saturated NaCl solution was then added until the vial was nearly full. Floating debris was removed using forceps, and more saturated NaCl solution was added until a convex meniscus formed at the rim of the vial. A coverslip was gently placed on the top of the vial, ensuring that no air bubbles were trapped. After standing for 10 min, the coverslip was carefully lifted and placed onto a glass slide.

Microscopic examination was performed by trained laboratory personnel using the same microscope, flotation procedure, and observation criteria for all samples. Formal blinding to treatment groups was not applied; therefore, the possibility of observer bias could not be completely excluded. To reduce subjectivity, the same examination procedure was applied consistently across all treatment groups and sampling times.

The slide was first screened under a light microscope at 10× magnification, and suspected coccidial oocysts were then confirmed at 40× magnification. For each sample, 10 non-overlapping microscopic fields were examined at 10× magnification before assigning the shedding intensity category. Oocyst shedding intensity was classified semi-quantitatively according to the average number of oocysts observed per microscopic field as follows: 1 to 3 oocysts per field, 1+; 4 to 6 oocysts per field, 2+; 7 to 10 oocysts per field, 3+; and more than 10 oocysts per field, 4+. Because this method was based on microscopic field observation, the results were interpreted as semi-quantitative fecal oocyst shedding intensity rather than oocysts per gram of feces.

The coccidial oocyst positive rate was calculated using the following formula:

$$Positive\ rate\ (\%) = \frac{Number\ of\ positive\ fecal\ samples}{Number\ of\ examined\ fecal\ samples} \times 100$$

The Willis flotation method was used as a practical field-based screening method to describe detectable fecal coccidial oocyst shedding patterns. McMaster counting was not performed in this study; therefore, the results should not be interpreted as quantitative OPG values or direct measurements of parasite burden. Because the flotation method detects oocysts but does not determine their viability, sporulation capacity, or infectivity, the parasitological results in this study were interpreted as detectable fecal coccidial oocyst shedding rather than confirmation of clinical coccidiosis.

Measurements

The main indicators recorded in this study included live body weight, body weight gain, feed intake, feed conversion ratio, survival rate, fecal coccidial oocyst positive rate, and semi-quantitative fecal coccidial oocyst shedding intensity. Because this study used fecal flotation to detect oocysts, the parasitological outcome was expressed as detectable fecal coccidial oocyst shedding rather than confirmed clinical coccidiosis or quantitative parasite burden.

The fecal coccidial oocyst positive rate was calculated as follows:

$$Positive\ rate\ (\%) = \frac{Number\ of\ positive\ fecal\ samples}{Number\ of\ examined\ fecal\ samples} \times 100$$

The shedding intensity was classified according to the average number of oocysts observed per microscopic field: 1 to 3 oocysts/field, 1+; 4 to 6 oocysts/field, 2+; 7 to 10 oocysts/field, 3+; and more than 10 oocysts/field, 4+.

Statistical analysis

Raw data were initially entered and checked using Microsoft Excel. Statistical analyses were performed using Minitab version 16.0. For growth performance variables, the replicate pen was considered the experimental unit. Body weight and body weight gain were first averaged within each replicate pen, and pen means were used for statistical analysis. Feed intake and feed conversion ratio were calculated at the replicate pen level. Growth performance parameters, including body weight, body weight gain, feed intake, and feed conversion ratio, were analyzed using the General Linear Model procedure. Normality and homogeneity of variance were checked using the Shapiro-Wilk test and Levene's test, respectively. When significant treatment effects were detected, treatment means were compared using Tukey's test at a 5% significance level. Results were presented as mean ± standard deviation (SD). Differences were considered statistically significant at $P<0.05$, and probability values originally displayed as 0.000 were presented as $P<0.001$.

Coccidial oocyst positive rate and oocyst shedding intensity categories were analyzed as categorical data. Differences among treatments at each sampling time were compared using the chi-square test. Fisher's exact test was used when the expected frequency in any cell was less than 5. Because multiple categorical comparisons were performed across age periods and shedding intensity categories, P values for oocyst data were interpreted cautiously, particularly borderline values. No formal Bonferroni correction was applied because these analyses were used to describe exploratory fecal oocyst shedding patterns across time rather than to test a single primary categorical endpoint. Results were presented as positive fecal samples/examined fecal samples with corresponding percentages.

For Tables 4 to 7, the denominators represent the number of valid fecal samples examined at each sampling time. In later sampling periods, the denominator in the control group decreased because two chickens died. No necropsy or laboratory diagnosis was performed; therefore, the cause of death was not confirmed, and possible mortality-related bias in later control-group estimates cannot be excluded. Survival rate was compared using Fisher's exact test because of the small number of mortality events.

RESULTS AND DISCUSSION

Effects of PCAW supplementation on body weight in Noi crossbred chickens

The effects of PCAW supplementation on body weight in Noi crossbred chickens are presented in Table 2. At 7 days of age, body weight was nearly identical among the four treatments, ranging from 61.30 to 61.33 g/chicken, with no significant difference among groups ($P>0.05$). This similarity was expected because chicks were selected for uniformity in age, clinical health status, and body weight before random allocation to treatments. Therefore, the experimental groups were comparable at the beginning of the feeding trial, and the difference observed at 70 days of age was unlikely to be explained by initial body weight variation.

Table 2: Effect of PCAW supplementation on body weight in Noi crossbred chickens (Mean ± SD).

Age, days	Treatment 1 control	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%	P value
7	61.33± 0.11	61.33± 0.10	61.30± 0.10	61.33 ± 0.10	0.967
70	885.33 ± 5.59^{c}	942.33± 5.92^{b}	998.67± 6.97^{a}	940.33 ± 5.59^{b}	<0.001

Means with different superscripts in the same row differ significantly ($P<0.05$). Values are presented as mean ± SD based on replicate pen means.

At 70 days of age, PCAW supplementation significantly affected final body weight ($P<0.001$). Chickens in the control group had the lowest final body weight, at 885.33 g/chicken, whereas all PCAW-supplemented groups showed higher values. The highest final body weight was recorded in the PCAW 0.6% group, reaching 998.67 g/chicken, which was 113.34 g/chicken higher than the control group. The PCAW 0.5% and PCAW 0.7% groups also had higher final body weights than the control group, at 942.33 and 940.33 g/chicken, respectively, but both were lower than the PCAW 0.6% group. The same superscript letter in the PCAW 0.5% and PCAW 0.7% groups indicates that these two groups did not differ significantly from each other. These findings show that PCAW supplementation was associated with improved final body weight, with the most favorable response observed at 0.6% under the conditions of this 70-day farm trial.

The body weight response did not increase linearly with PCAW concentration. Although PCAW 0.5% and PCAW 0.7% both improved final body weight relative to the control group, neither reached the response observed at 0.6%. This pattern suggests that the 0.6% concentration may have provided a more suitable practical level than either the lower or higher concentration tested. The lower response at 0.7% compared with 0.6% may indicate that increasing the concentration beyond a moderate level did not provide additional growth benefit. Because water intake, palatability, residual alcohol exposure, and physiological tolerance were not directly evaluated, the reason for the lower response at 0.7% cannot be confirmed.

The higher final body weight observed in the PCAW-supplemented groups may be discussed in relation to the

plant materials included in PCAW, but the interpretation must remain cautious. Garlic contains sulfur-containing compounds, particularly alliin and allicin, which have been associated in previous studies with antimicrobial activity, digestive support, and improved gut function. Williams *et al.* (2001) suggested that improved nutrient availability associated with garlic supplementation may enhance feed utilization efficiency by supporting mineral and vitamin balance in the diet and promoting the colonization of beneficial microorganisms in the gastrointestinal tract. In the present study, however, nutrient digestibility, gut microbiota, intestinal lesions, and immune markers were not measured. Therefore, the role of garlic-derived compounds in the observed growth response remains a possible explanation rather than a mechanism confirmed by the present data.

Lemongrass may also provide a possible biological basis for the performance response observed in the PCAW-supplemented groups. Lemongrass has been suggested as a promising natural alternative to antibiotics in poultry production (Khan *et al.*, 2022; Abd El-Ghany *et al.*, 2022). Its essential oil contains bioactive components such as citral and myrcene, which may contribute to antimicrobial and digestive-supporting effects. Essential oils may also influence physiological responses, including thyroid hormone activity, which is associated with metabolic regulation and growth (El-Hady *et al.*, 2020). In addition, lemongrass has been reported to exert antihypertensive effects in animals, potentially through stimulation of parasympathetic activity (Silva and Bárbara, 2022). These previous reports support possible biological explanations for the improved body weight response, but endocrine, cardiovascular, intestinal, and microbial parameters were not evaluated in the present study. Therefore, these mechanisms should be considered hypotheses requiring further investigation.

Guava leaves may further contribute to the possible biological activity of PCAW because they contain tannins and phenolic compounds. These compounds have been associated with antioxidant, antimicrobial, and intestinal-supporting properties in previous studies. Langerudi *et al.* (2022) reported that supplementation with *Psidium guajava* essential oil, particularly at 5 mg/kg feed, may help prevent coccidiosis and improve health status in broiler chickens. However, the present study used guava leaves as part of a locally prepared wine infusion rather than as an essential oil. Therefore, this citation was used only to support the broader biological potential of *Psidium guajava*-derived products, not as direct evidence that the PCAW preparation has the same chemical composition or anticoccidial effect as guava essential oil.

Overall, the body weight data in Table 2 should be interpreted as initial and final body weight responses rather than a full growth curve. Intermediate body weights at 21, 35, and 49 days were not recorded; therefore, the exact time at which the PCAW effect on growth began could not be determined. The present findings indicate that PCAW 0.6% produced the highest final body weight under the conditions of this 70-day single-farm trial. However, because this study did not include serial body weight measurements, water intake records, residual alcohol assessment, phytochemical characterization of PCAW, gut lesion scoring, gut histology, immune markers, or microbiome analysis, the biological mechanism underlying the growth response cannot be confirmed. Further studies with larger numbers of replicate pens, repeated farm conditions, serial body weight measurements, chemical characterization of PCAW, residual alcohol assessment, and direct intestinal and physiological measurements are needed before broader recommendations can be made.

Effects of PCAW supplementation on feed conversion ratio in Noi crossbred chickens

The effects of PCAW supplementation on body weight gain, feed intake, and feed conversion ratio (FCR) in Noi crossbred chickens are shown in Table 3. Body weight gain differed significantly among treatments ($P<0.001$), whereas feed intake did not differ among groups ($P=0.981$). The control group had the lowest body weight gain, at 824.00 g/chicken, whereas the highest value was recorded in the PCAW 0.6% group, at 937.37 g/chicken. Feed intake was similar across treatments, ranging from 2818.08 to 2828.01 g/chicken. This finding is important because it indicates that the improvement in FCR was mainly associated with greater body weight gain from a comparable amount of feed, rather than increased feed intake.

Table 3: Body weight gain, feed intake, and feed conversion ratio of Noi crossbred chickens supplemented with different levels of PCAW (Mean ± SD).

Item	Treatment 1 control	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%	P value
Body weight gain, g/chicken	824.00 ± 5.51^{c}	881.00 ± 5.84^{b}	937.37 ± 6.88^{a}	879.00 ± 5.51^{b}	<0.001
Feed intake, g/chicken	2818.08 ± 73.45	2828.01 ± 69.72	2821.48 ± 71.36	2821.59 ± 70.84	0.981
FCR	3.42 ± 0.08^{a}	3.21 ± 0.06^{b}	3.01 ± 0.05^{c}	3.21 ± 0.07^{b}	<0.001

Means with different superscripts in the same row differ significantly ($P<0.05$). Values are presented as mean ± SD based on replicate pen means. FCR = feed conversion ratio; PCAW = *Psidium guajava, Cymbopogon citratus,* and *Allium sativum* wine infusion. Feed intake and FCR were calculated at the replicate pen level.

FCR differed significantly among treatments (P<0.001). The control group had the highest FCR, at 3.42, while all PCAW-supplemented groups showed lower FCR values. The most favorable FCR was observed in the PCAW 0.6% group, at 3.01. The PCAW 0.5% and PCAW 0.7% groups had similar FCR values, both at 3.21, which were lower than the control group but higher than the PCAW 0.6% group. Because feed intake and FCR were calculated at the replicate pen level using total feed intake and body weight gain, the mean ± SD values in Table 3 represent variation among replicate pen means. Therefore, the FCR result should be interpreted as a pen-level performance response under the present experimental conditions.

The absence of a significant difference in feed intake is important for interpreting the FCR response. Because chickens in all treatments consumed comparable amounts of feed, the lower FCR in the PCAW 0.6% group appears to reflect greater weight gain from a similar amount of feed. This partly addresses the concern that PCAW might have changed feed consumption through palatability-related effects. However, water intake was not measured, and PCAW contained garlic and was prepared using wine. Therefore, possible effects on drinking behavior, actual PCAW consumption, palatability, and physiological tolerance cannot be excluded. These factors should be evaluated in future studies.

Improved feed conversion is an important target in poultry production because efficient nutrient use supports growth while reducing feed cost. Ogbuewu *et al*. (2019) emphasized that a major objective of modern poultry production is to improve nutrient utilization and growth performance while maintaining efficient feed intake. In this context, plant-based feed additives have attracted attention because they may reduce dependence on commercial additives and provide a cost-effective alternative to synthetic growth promoters (Basit *et al*., 2025). The present result is consistent with this general concept because PCAW 0.6% was associated with improved FCR without increasing feed intake. However, the present study was not designed to confirm the physiological mechanism responsible for this response.

The improvement in FCR may be discussed in relation to the plant materials included in PCAW, but this interpretation should remain cautious. Garlic contains sulfur-containing compounds, particularly alliin and allicin, which have been associated with antimicrobial activity and digestive support in previous studies. According to Asquith and Butler (1986), the formation of a curdy white precipitate during tannin determination indicates the presence of tannins in guava leaf extract. The presence of tannins and other phenolic compounds in guava leaves may partly explain their reported antioxidant, antimicrobial, antifungal, antidiarrheal, anti-inflammatory, and antidiabetic effects. However, the present study did not measure allicin, tannins, total phenolics, gut microbiota, nutrient digestibility, or intestinal lesions. Therefore, the contribution of these compounds to the observed FCR response remains a possible explanation rather than a mechanism confirmed by the present experiment.

The relationship between coccidial exposure and feed efficiency is also relevant to the present findings. Sharman *et al*. (2010) reported that *Eimeria* infection can reduce feed efficiency, mainly because the endogenous stages of the parasite disrupt intestinal integrity and impair digestive function. In the present study, PCAW-supplemented groups showed lower detectable fecal coccidial oocyst shedding than the control group. This pattern may be associated with the better FCR observed in the PCAW 0.6% group. However, intestinal lesion scores, gut histology, gut integrity, immune markers, and *Eimeria* species identification were not evaluated. Therefore, the improved FCR cannot be attributed directly to reduced intestinal damage or reduced coccidial infection. The result should instead be interpreted as an association among PCAW supplementation, lower detectable fecal coccidial oocyst shedding, higher body weight gain, similar feed intake, and improved FCR.

The response was not linear with increasing PCAW concentration. Although PCAW 0.5% and PCAW 0.7% improved FCR compared with the control group, neither reached the response observed at 0.6%. This pattern suggests that PCAW may have a practical effective range, in which a moderate inclusion level provides a more favorable response than either a lower or higher level. The lack of further improvement at 0.7% may indicate that increasing the concentration beyond 0.6% did not provide additional benefit for feed utilization. Palatability, drinking behavior, residual alcohol exposure, and physiological tolerance were not directly evaluated, and these factors may partly explain why the response did not increase at the highest PCAW level.

The data in Table 3 indicate that PCAW supplementation, particularly at 0.6%, was associated with improved FCR in Noi crossbred chickens. Because feed intake did not differ significantly among treatments, the better FCR in the PCAW 0.6% group was mainly related to higher body weight gain. Nevertheless, this result should be interpreted as a practical farm-based performance outcome under the conditions of this single-farm trial, not as proof of a specific digestive, microbial, or anticoccidial mechanism. The number of replicate pens was limited, and no formal power analysis was performed before the experiment. Therefore, future studies with more replicate pens, water intake records, phytochemical characterization of PCAW,

residual alcohol quantification, controlled alcohol-only treatments, and direct intestinal measurements are needed to confirm the consistency and biological basis of the FCR response.

Effects of PCAW supplementation on coccidial oocyst shedding in experimental chickens

Fecal oocyst shedding has been widely used as a practical indicator for assessing the level of *Eimeria* infection in chickens (Du *et al*., 2005; Talebi and Mulcahy, 2005; Lee *et al*., 2009). In the present study, the effects of PCAW supplementation on detectable fecal coccidial oocyst shedding across the experimental period are presented in Table 4. Coccidial oocysts were detected from the early stage of the experiment, indicating that chickens were exposed to natural coccidial infection pressure under farm conditions. *Eimeria* oocysts are widely distributed in poultry environments and can spread rapidly within farms. Williams (1995) reported that the sporulation of coccidial oocysts can be influenced by decomposition, bacterial damage, and ammonia released from litter, which may affect oocyst survival in the environment. Their environmental resistance and tolerance to several disinfectants make coccidial control difficult, especially under poor litter hygiene or high infection pressure (López-Osorio *et al*., 2020; Attree *et al*., 2021).

As shown in Table 4, the fecal coccidial oocyst positive rate differed among treatments at all sampling periods ($P<0.05$). The control group consistently showed the highest positive rate, whereas the PCAW-supplemented groups generally had lower values. At 8 to 14 days of age, 9/30 samples in the control group were positive, corresponding to 30.00%, whereas no positive sample was detected in the PCAW 0.6% group. This result should be interpreted as no oocysts detected by the flotation method, not as proof that infection was completely absent. From 15 to 21 days onward, the difference between the control group and the PCAW 0.6% group became clearer. The control group reached 50.00% at 15 to 21 days and 63.33% at 29 to 35 days, while the PCAW 0.6% group remained lower, ranging from 3.33% to 13.33% during the same period. At 64 to 70 days of age, the control group still showed 16/28 positive samples, or 57.14%, whereas the PCAW 0.6% group showed only 1/30 positive sample, or 3.33%. These findings indicate that PCAW supplementation, particularly at 0.6%, was associated with a lower detectable fecal oocyst positive rate under the conditions of this farm trial.

Most chickens are exposed to coccidia during their lifetime, but infection does not always develop into clinical coccidiosis. Clinical disease is more frequently observed in young chickens, which are more susceptible to intestinal damage and performance loss, although adult chickens may also be affected under certain conditions (Kendall, 1972; Chartier and Paraud, 2012; Chapman, 2014). Shirzad *et al*. (2011) reported that subclinical coccidiosis is closely associated with flock age, with the highest frequency commonly observed in chickens at 5 to 6 weeks of age. The higher positive rate in the control group around 29 to 35 days of age is consistent with this age-related susceptibility. In chickens, seven *Eimeria* species have been described, among which *E. tenella*, *E. maxima*, and *E. acervulina* are considered the most economically important because of their pathogenic effects on intestinal health and productivity (Thenmozhi *et al*., 2014). Mixed infection with multiple *Eimeria* species is also common in chicken coccidiosis and may increase disease severity while complicating accurate diagnosis (Haug *et al*., 2008; Jenkins *et al*., 2008). Because *Eimeria* species identification was not performed in the present study, the findings should be interpreted as changes in detectable fecal coccidial oocyst shedding rather than species-specific efficacy or confirmed protection against clinical coccidiosis.

Table 4: Effect of PCAW supplementation on fecal coccidial oocyst positive rate across experimental weeks in Noi crossbred chickens.

Age, days	Treatment 1 control	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%	P value
8 to 14	9/30 (30.00)	4/30 (13.33)	0/30 (0.00)	6/30 (20.00)	0.006
15 to 21	15/30 (50.00)	4/30 (13.33)	1/30 (3.33)	5/30 (16.67)	<0.001
22 to 28	16/30 (53.33)	6/30 (20.00)	3/30 (10.00)	7/30 (23.33)	0.001
29 to 35	19/30 (63.33)	5/30 (16.67)	4/30 (13.33)	7/30 (23.33)	<0.001
36 to 42	14/28 (50.00)	6/30 (20.00)	3/30 (10.00)	6/30 (20.00)	0.003
43 to 49	13/28 (46.43)	4/30 (13.33)	2/30 (6.67)	7/30 (23.33)	0.002
50 to 56	13/28 (46.43)	5/30 (16.67)	1/30 (3.33)	7/30 (23.33)	<0.001
57 to 63	15/28 (53.57)	5/30 (16.67)	2/30 (6.67)	6/30 (20.00)	<0.001
64 to 70	16/28 (57.14)	4/30 (13.33)	1/30 (3.33)	7/30 (23.33)	<0.001

Values are presented as positive fecal samples/examined fecal samples (positive rate, %). The denominators refer to the number of valid fecal samples examined at each sampling time. Differences among treatments at each age period were tested using the chi-square test or Fisher's exact test when expected cell counts were less than 5.

Table 5: Effect of PCAW supplementation on 1+ fecal coccidial oocyst shedding intensity in Noi crossbred chickens.

Age, days	Treatment 1 control	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%	P value
8 to 14	5/30 (16.67)	3/30 (10.00)	0/30 (0.00)	5/30 (16.67)	0.082
15 to 21	12/30 (40.00)	4/30 (13.33)	1/30 (3.33)	4/30 (13.33)	0.001
22 to 28	13/30 (43.33)	5/30 (16.67)	3/30 (10.00)	6/30 (20.00)	0.013
29 to 35	13/30 (43.33)	3/30 (10.00)	3/30 (10.00)	5/30 (16.67)	0.003
36 to 42	10/28 (35.71)	5/30 (16.67)	3/30 (10.00)	5/30 (16.67)	0.081
43 to 49	10/28 (35.71)	3/30 (10.00)	2/30 (6.67)	6/30 (20.00)	0.025
50 to 56	10/28 (35.71)	5/30 (16.67)	1/30 (3.33)	6/30 (20.00)	0.017
57 to 63	11/28 (39.29)	5/30 (16.67)	2/30 (6.67)	5/30 (16.67)	0.015
64 to 70	13/28 (46.43)	3/30 (10.00)	1/30 (3.33)	6/30 (20.00)	<0.001

Values are presented as positive fecal samples/examined fecal samples (percentage, %). The 1+ category indicates 1 to 3 oocysts per microscopic field. Differences among treatments at each age period were tested using the chi-square test or Fisher's exact test when expected cell counts were less than 5.

The lower fecal oocyst positive rate in PCAW-supplemented groups may be discussed in relation to the plant materials included in PCAW, but the interpretation must remain cautious. Garlic contains sulfur-containing compounds such as alliin and allicin, which have been associated in previous studies with antimicrobial and antiparasitic properties. Guava leaves contain tannins and phenolic compounds, and lemongrass contains essential oil components, including citral-related compounds. These plant-derived compounds may provide possible biological explanations for the lower detectable oocyst shedding observed in PCAW-supplemented groups. However, allicin, citral, tannins, total phenolics, intestinal lesion scores, gut histology, immune markers, microbiota, oocyst viability, sporulation capacity, and *Eimeria* species were not evaluated in the present study. Therefore, the reduction in fecal oocyst shedding should be interpreted as an observed parasitological response rather than direct evidence of parasite killing, immune stimulation, improved gut integrity, or prevention of coccidiosis.

The 1+ fecal coccidial oocyst shedding intensity is shown in Table 5. The control group generally showed a higher proportion of 1+ positive samples than the PCAW 0.6% group. Significant treatment differences were detected at several sampling periods from 15 to 21 days onward, whereas the differences at 8 to 14 days and 36 to 42 days were not statistically significant. At 29 to 35 days, the control group had 13/30 samples at 1+ intensity, or 43.33%, whereas the PCAW 0.6% group had 3/30 samples, or 10.00%. By 64 to 70 days, the control group still had 13/28 samples at 1+ intensity, or 46.43%, compared with 1/30 sample, or 3.33%, in the PCAW 0.6% group. These data indicate that PCAW 0.6% was associated with a lower occurrence of low-level detectable oocyst shedding across several sampling periods.

The reduction in 1+ shedding is relevant because even low-intensity oocyst shedding can contribute to environmental contamination and maintain infection pressure in litter-based systems. Warm and humid environmental conditions can promote oocyst sporulation and facilitate coccidial transmission. Farm-level factors, particularly litter management and biosecurity practices, also play an important role in determining oocyst survival, environmental contamination, and parasite dissemination within poultry flocks (Wondimu *et al*., 2019; Pajíc *et al*., 2023). Therefore, reducing the number of positive fecal samples, even at 1+ intensity, may help lower environmental oocyst accumulation. This interpretation should remain cautious because oocyst viability, sporulation ability, and infectivity were not assessed.

The 2+ fecal coccidial oocyst shedding intensity is presented in Table 6. Moderate shedding was numerically more frequent in the control group than in the PCAW 0.6% group at several sampling periods. However, most comparisons for 2+ intensity were not statistically significant (P>0.05), except at 57 to 63 days (P=0.049). Because multiple categorical comparisons were performed across sampling periods and intensity categories, this borderline result should be interpreted cautiously. At 8 to 14 days of age, the control group had 4/30 samples at 2+ intensity, or 13.33%, whereas no 2+ sample was detected in the PCAW 0.6% group. At 29 to 35 days, when the overall positive rate was highest in the control group, 2+ intensity was detected in 4/30 control samples, or 13.33%, compared with 1/30 sample, or 3.33%, in the PCAW 0.6% group. From 36 to 42 days onward, no 2+ sample was detected in the PCAW 0.6% group. These findings suggest a lower numerical occurrence of moderate shedding in the PCAW 0.6% group, but the statistical evidence for this intensity category was limited.

Table 6: Effect of PCAW supplementation on 2+ fecal coccidial oocyst shedding intensity in Noi crossbred chickens.

Age, days	Treatment 1 control	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%	P value
8 to 14	4/30 (13.33)	1/30 (3.33)	0/30 (0.00)	1/30 (3.33)	0.165
15 to 21	2/30 (6.67)	0/30 (0.00)	0/30 (0.00)	1/30 (3.33)	0.611
22 to 28	2/30 (6.67)	1/30 (3.33)	0/30 (0.00)	1/30 (3.33)	0.902
29 to 35	4/30 (13.33)	2/30 (6.67)	1/30 (3.33)	2/30 (6.67)	0.629
36 to 42	3/28 (10.71)	1/30 (3.33)	0/30 (0.00)	1/30 (3.33)	0.218
43 to 49	2/28 (7.14)	1/30 (3.33)	0/30 (0.00)	1/30 (3.33)	0.465
50 to 56	2/28 (7.14)	0/30 (0.00)	0/30 (0.00)	1/30 (3.33)	0.181
57 to 63	3/28 (10.71)	0/30 (0.00)	0/30 (0.00)	1/30 (3.33)	0.049
64 to 70	2/28 (7.14)	1/30 (3.33)	0/30 (0.00)	1/30 (3.33)	0.475

Values are presented as positive fecal samples/examined fecal samples (percentage, %). The 2+ category indicates 4 to 6 oocysts per microscopic field. Differences among treatments at each age period were tested using Fisher's exact test because of low expected cell counts.

Table 7: Effect of PCAW supplementation on 3+ fecal coccidial oocyst shedding intensity in Noi crossbred chickens.

Age, days	Treatment 1 control	Treatment 2 PCAW 0.5%	Treatment 3 PCAW 0.6%	Treatment 4 PCAW 0.7%	P value
8 to 14	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	-
15 to 21	1/30 (3.33)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	1.000
22 to 28	1/30 (3.33)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	1.000
29 to 35	2/30 (6.67)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0.250
36 to 42	1/28 (3.57)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0.235
43 to 49	1/28 (3.57)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0.237
50 to 56	1/28 (3.57)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0.232
57 to 63	1/28 (3.57)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0.230
64 to 70	1/28 (3.57)	0/30 (0.00)	0/30 (0.00)	0/30 (0.00)	0.239

Values are presented as positive fecal samples/examined fecal samples (percentage, %). The 3+ category indicates 7 to 10 oocysts per microscopic field. No 4+ intensity samples were detected in any treatment during the experimental period. P value was not calculated for 8 to 14 days because no 3+ samples were detected in any treatment. Differences among treatments were tested using Fisher's exact test. PCAW = *Psidium guajava, Cymbopogon citratus,* and *Allium sativum* wine infusion.

The 2+ results should therefore be interpreted as supportive descriptive evidence rather than a strong quantitative measure of parasite burden. Moderate oocyst shedding may contribute to environmental contamination and may reflect more active parasite cycling within the flock. However, the flotation method used in this study did not quantify oocysts per gram of feces and did not determine whether detected oocysts were viable, sporulated, or infective. In addition, the small number of samples in the 2+ category reduced statistical power. For this reason, conclusions based on 2+ intensity should remain cautious.

The 3+ fecal coccidial oocyst shedding intensity is shown in Table 7. No 3+ sample was detected in any PCAW-supplemented group throughout the experimental period. In contrast, 3+ samples were detected only in the control group from 15 to 21 days onward, with the highest value observed at 29 to 35 days, reaching 2/30 samples, or 6.67%. However, the differences among treatments were not statistically significant for this category (P>0.05), and the low number of 3+ samples limits interpretation. Therefore, the absence of 3+ samples in PCAW-supplemented groups should be described as a favorable numerical pattern, not as proof that PCAW prevented high-intensity shedding.

A decrease in fecal oocyst excretion is generally regarded as an indication of reduced coccidial infection intensity and lower parasite burden in chickens (Küçükyilmaz *et al*., 2012). In the present study, this interpretation should be applied carefully because the flotation method was semi-quantitative and did not include OPG determination. The method detected coccidial oocysts but could not distinguish viable and infective oocysts from dead, damaged, empty, or non-infective oocysts. It also could not exclude the possibility that PCAW affected oocyst distribution, clumping, adhesion to fecal material, or recovery during microscopic examination. Therefore, lower detected oocyst numbers in PCAW-supplemented groups should be interpreted as reduced detectable fecal coccidial oocyst shedding rather than direct proof of reduced parasite replication or complete oocyst inactivation.

Taken together, Tables 4, 5, 6, and 7 indicate that PCAW supplementation was associated with a lower fecal oocyst positive rate and a generally lower semi-quantitative shedding profile, particularly at the 0.6% concentration. The strongest statistical support was observed for the overall oocyst positive rate in Table 4 and for several 1+ comparisons in Table 5. In contrast, the 2+ and 3+ intensity categories contained fewer positive samples and therefore provided weaker statistical evidence. For this reason, the most appropriate conclusion is that PCAW 0.6% was associated with reduced detectable fecal coccidial oocyst shedding under the present farm conditions. These results should not be interpreted as evidence that PCAW prevented or treated clinical coccidiosis. Future studies should include McMaster OPG quantification, blinded microscopic examination, oocyst viability assessment, sporulation tests, intestinal lesion scoring, and *Eimeria* species identification to clarify whether PCAW reduces parasite replication, affects oocyst recovery, or changes the viability and infectivity of shed oocysts.

Survival rate

The survival rate of Noi crossbred chickens in the different experimental treatments is presented in Table 8. The control group started with 40 chickens and ended with 38 chickens, corresponding to a survival rate of 95.00%. In contrast, all PCAW-supplemented groups maintained 40 chickens until the end of the experiment, giving a survival rate of 100.00%. However, this numerical difference was not statistically significant by Fisher's exact test (P=0.49, two-tailed). Therefore, the survival result should be interpreted as a descriptive observation under the present farm conditions rather than evidence of a significant survival-promoting effect of PCAW.

Table 8: Effect of PCAW supplementation on survival rate in Noi crossbred chickens.

Treatment	Initial number of chickens	Final number of chickens	Survival rate (%)
Treatment 1 (0%)	40	38	95.00
Treatment 2 PCAW (0.5%)	40	40	100.00
Treatment 3 PCAW (0.6%)	40	40	100.00
Treatment 4 PCAW (0.7%)	40	40	100.00

Survival rate was calculated as final number of chickens/initial number of chickens × 100. The survival difference between each PCAW-supplemented group and the control group was not statistically significant by Fisher's exact test (P=0.49, two-tailed). No necropsy was performed; therefore, the cause of death in the control group was not confirmed.

The absence of mortality in the PCAW-supplemented groups may indicate favorable flock stability during the 70-day farm trial, but it should not be interpreted as proof that PCAW prevented death from coccidiosis. Mortality associated with coccidial infection is closely related to infection severity (Christaki *et al*., 2004). In the present study, PCAW-supplemented groups, especially PCAW 0.6%, showed lower detectable fecal coccidial oocyst shedding than the control group. However, clinical coccidiosis, intestinal lesions, cause-specific mortality, and necropsy findings were not evaluated. Therefore, the survival data should be viewed only as a descriptive flock outcome, not as direct evidence of protection against clinical coccidiosis or mortality reduction.

The numerical survival pattern may be discussed in relation to the potential biological properties of the herbal components in PCAW, but this interpretation remains speculative. Adaszyńska-Skwirzyńska and Szczerbińska (2017) reported that herbs and plant-derived compounds may exhibit antimicrobial, antioxidant, and anti-inflammatory activities and may help reduce the severity of coccidial infection in poultry. However, the present study did not assess immune markers, blood parameters, gut histology, microbiota, inflammatory responses, or confirmed cause of death. Therefore, these mechanisms cannot be confirmed from the present data.

The practical use of herbal preparations also requires careful consideration. Sen and Chakraborty (2017) emphasized that the safety and efficacy of herbal medicines require further scientific validation. Calixto (2019) also highlighted that the lack of standardized extraction methods and dosing protocols can lead to variation in bioactive compound concentration and inconsistent biological responses. These concerns are relevant to the present study because PCAW was prepared as a locally practical herbal wine infusion rather than as a chemically standardized extract. In addition, residual alcohol concentration in drinking water was not measured, and an alcohol-only control group was not included. Thus, the observed survival pattern cannot be attributed exclusively to the herbal components.

Taken together, Table 8 shows that all PCAW-supplemented groups maintained 100.00% survival during the 70-day trial, whereas the control group had 95.00% survival. However, because mortality was low, the difference was not statistically significant, and no necropsy was performed, the survival result should be interpreted as a favorable numerical observation under the present farm conditions rather than a conclusive survival benefit of PCAW.

CONCLUSIONS

Under the conditions of this 70-day single-farm trial, PCAW supplementation at 0.6% produced the most

favorable response in Noi crossbred chickens, with the highest final body weight, improved feed conversion ratio, and lower detectable fecal coccidial oocyst shedding. Feed intake did not differ among treatments, and the survival difference was not statistically significant. The practical take-home message is that PCAW 0.6% may be considered a supportive drinking-water supplement for improving growth performance and reducing detectable fecal oocyst shedding under similar farm conditions. However, PCAW should not be regarded as a confirmed preventive or therapeutic treatment for coccidiosis until further controlled studies are conducted.

ACKNOWLEDGEMENT

We acknowledge the support of time and facilities from Vinh Long University of Technology Education for this study.

NOVELTY STATEMENT

This study provides preliminary evidence on the use of a locally prepared herbal wine infusion made from *Psidium guajava*, *Cymbopogon citratus*, and *Allium sativum* as a practical plant-based supplement for Noi crossbred chickens. The novelty of this work lies in evaluating a combined herbal wine infusion administered through drinking water under farm conditions, rather than assessing a single plant material or a chemically standardized extract. The findings indicate that PCAW, particularly at 0.6%, was associated with improved final body weight, better feed conversion ratio, and reduced detectable fecal coccidial oocyst shedding. However, these effects should be interpreted as preliminary performance and parasitological observations, not as confirmed prevention or treatment of clinical coccidiosis. Further studies with phytochemical characterization, residual alcohol assessment, OPG quantification, oocyst viability testing, and *Eimeria* species identification are needed to validate and standardize PCAW for wider application.

AUTHORS' CONTRIBUTION

Quach Thi Thanh Tam: conceptualization, methodology, data curation and writing original draft. Truong Phuc Vinh: conceptualization, resources and formal analysis. Vo Thi Ngoc Bich: software, and writing original draft. Phan Nhan: visualization, and writing review and editing. All authors reviewed and approved the final manuscript.

Ethical approval

This study involved routine farm management and non-invasive fecal sample collection from chickens reared under farm conditions. No surgical procedures, blood collection, experimental infection, force-feeding, or necropsy were performed. Therefore, no formal animal ethics approval number was issued for this study. The experiment was conducted with permission from the farm owner and followed routine poultry management, vaccination, and health monitoring practices recommended by the Department of Animal Husbandry, Veterinary Medicine and Fisheries of Vinh Long Province. Animal handling was minimized, and all chickens were monitored daily for health and welfare throughout the experimental period. The study was also conducted in accordance with the animal husbandry and animal care principles of the Vietnamese Law on Animal Husbandry No. 32/2018/QH14.

Generative AI and AI-assisted technology statement

Generative AI tools were not used to generate any scientific content. Any AI assistance was limited to minor language editing, and all ideas, interpretations, and conclusions are solely those of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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