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Research article

Effects of supplementing of protease and hydrolyzed tannin on growth performance, meat yield and coccidia infection of Japanese quail

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Abstract

This study investigated the impact of dietary protease and hydrolyzed tannin supplementation on growth performance, meat yield and coccidia infection in Japanese quail. A total of 550 one-day-old quail were randomly assigned to five dietary treatments, with each treatment replicated four times (27-28 birds/replicate). The treatments included: a control (basal diet only); the basal diet supplemented with P125 (125 mg protease/kg feed), HT300 (300 mg tannin/kg feed), HT150P125 (150 mg tannin/kg feed + 125 mg protease/kg feed), and HT300P125 (300 mg tannin/kg feed + 125 mg protease/kg feed). Survival rates were numerically higher in the HT300 and HT150P125 groups than in the control and HT300P125 groups ($p>0.05$). While body weight, average daily gain, and feed intake did not significantly differ among treatments ($p>0.05$), the feed conversion ratio was significantly affected from 1 to 42 days ($p<0.05$). Supplementation with combined additives significantly increased relative breast percentage and breast meat yield ($p>0.05$), reaching peak values of 46.6% and 32.2%, respectively, in the HT300P125 group. Fecal oocyst shedding showed non-significant numerical reductions in the P125, HT150P125, and HT300P125 groups compared to the control ($p > 0.05$). In conclusion, dietary supplementation with hydrolyzed tannin at 300 mg/kg enhances feed conversion efficiency, whereas co-supplementation with 300 mg/kg hydrolyzed tannin and 125 mg/kg protease synergistically maximizes breast muscle yield in Japanese quail.

Keywords: Coccidia infection, Growth performance, Hydrolyzed tannin, Protease, Japanese quail

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INTRODUCTION

Japanese quail (*Coturnix japonica*) production is highly valued for its rapid turnover and economic efficiency in both the meat and egg sectors. Although formal commercial census data regarding quail farming in Vietnam remain limited, the widespread distribution of quail products across domestic markets reflects a steadily growing consumer demand. However, the intensive rearing practices required to sustain market demand inevitably increase bird density, thereby elevating the risk of enteric infections and intestinal disorders.

Avian coccidiosis remains one of the most prevalent and economically impactful diseases in the global poultry industry, with recent studies reporting prevalence rates as high as 46.1% in Japanese quail populations (Tran et al., 2025). Due to their exceptionally high metabolic rate, Japanese quail are particularly vulnerable to severe performance losses when enteric health and nutrient absorption are compromised. Consequently, preserving intestinal integrity is vital to maintaining the economic viability of quail production. To address these challenges, bioactive feed additives such as plant-derived polyphenols, particularly tannins, have gained significant attention for their potent antimicrobial, anti-inflammatory, and gut-health-promoting properties (Choi and Kim, 2020). Specifically, hydrolyzable tannin (HT) has been shown to effectively alleviate oxidative stress, reduce intestinal inflammation, and protect the mucosal barrier of the jejunum (Zhang et al., 2024). Furthermore, dietary supplementation with 0.2% HT has been reported to increase daily feed intake and average daily gain while reducing nitrogen content in broiler litter (Schiavone et al., 2008). Complementary to the protective pathways of tannins, addressing infection-induced malabsorption is equally critical. Exogenous protease supplementation improves feed conversion, accelerates amino acid digestibility, and positively modulates intestinal morphology by enhancing villus height and enterocyte proliferation (Cardinal et al., 2019). By breaking down resilient dietary proteins, proteases minimize the volume of undigested protein substrates reaching the hindgut, effectively depriving pathogenic microbes of their primary fermentation sources. Consequently, exogenous protease supplementation has been shown to alleviate performance losses during subclinical coccidiosis in broilers (Peek et al., 2009) and enhance growth in meat-type quail fed low-protein formulations (Diana et al., 2020).

Although individual applications of proteases and tannins have been evaluated—predominantly in broiler chickens—their potential synergistic effects in Japanese quail remain unexamined. We hypothesized that combining exogenous protease with hydrolyzed tannins would provide a dual-action, antibiotic-free strategy to enhance growth performance, improve carcass yield, and bolster resilience against enteric coccidial challenge.

MATERIALS AND METHODS

Animals, housing and care

The experiment was conducted at the experimental farm of the Faculty of Animal Sciences, Can Tho University, Viet Nam. The quail were housed in wired-mesh cages equipped with underlying collection trays. The ambient temperature within the house ranged from 28°C to 33°C, with a relative humidity between 67% and 89%. Each cage was fitted with a feeder and an automatic drinking system. Heat lamps were utilized for brooding from day 1 to day 21. Feed and water were provided *ad libitum*, with feed replenished twice daily (08:00 and 15:00). All experimental animal procedures were approved by the Animal Ethics Committee of Can Tho University (Approval No. CTU-AEC24003).

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Experimental design

The experiment randomly arranged 550 one-day-old quail to five dietary treatments, each with four replicates of 27-28 birds. The treatments included: (1) a control (basal diet only), and (2) the basal diet supplemented with 125 mg protease/kg feed (P125), (3) 300 mg hydrolyzed tannin/kg feed (HT300), (4) 150 mg hydrolyzed tannin/kg feed plus 125 mg protease/kg feed (HT150P125), and (5) 300 mg hydrolyzed tannin/kg feed plus 125 mg protease/kg feed (HT300P125).

The feeding program consisted of two phases: a starter phase (days 1 to 21) and a finisher phase (days 22 to 42). Commercially manufactured mash formulations were used as the basal diets. The starter basal diet contained 2,800 kcal/kg of metabolizable energy (ME) and 20% crude protein (CP), while the finisher diet contained 2,825 kcal/kg ME and 18% CP. The proximate chemical and nutritional compositions of the basal diets were analyzed prior to the trial. The experimental additives (protease and hydrolyzed tannin) were throughly homogenized into the basal diet daily before feeding.

The exogenous protease used in this study was a fine, light-brown powder (Jefo Protease®, Jefo Nutrition Inc., Canada). The hydrolyzed tannin source was a commercial chestnut extract containing 75% tannins, supplied as a fine, reddish-brown powder, water-soluble powder. All commercial basal feeds, and additives were purchased from local suppliers.

Data collection

The body weight (BW) of the experimental quail was recorded on day 1, and subsequently at weekly intervals until day 42. Feed intake (FI) per replicate was recorded daily to calculate average daily gain (ADG) and feed conversion ratio (FCR). Between 28 and 42 days of age, 2-5 g of freshly voided feces were collected from each replicate and pooled at three-day intervals. The fooled fecal samples were stored at 5oC prior to parasite analysis. Coccidial oocysts were identified and enumerated using standard floatation in a saturated sodium chloride solution (specific gravity = 1.20 g/mL) (Willis, 1921).

On day 42, eight birds per treatment approximating the mean BW of their respective treatment group were selected for carcass trait evaluations. Prior to slaughter, the selected birds were subjected to a 12-hour feed withdrawal period with *ad libitum* access to water. The birds were re-weighed immediately before being slaughtered via cervical dislocation. Carcass yield and the relative weights of the breast, thighs, and visceral organs (gizzard, heart, and liver) were recorded and calculated as a percentage of the hot carcass weight (Khang et al., 2024).

Statistical analysis

Data were compiled using Microsoft Excel and subjected to statistical analyzed using Minitab software (version 16.0). Growth performance and carcass traits were evaluated using one-way analysis of variance (ANOVA) via the General Linear Model (GLM) procedure. Differences among treatment means were determined using Tukey's post-hoc test, with statistical significance declared at $p<0.05$. Furthermore, categorical variables, including survival rate and oocyst infection rate, were analyzed using the Chi-square test (χ^2).

RESULTS

Growth performance

No statistically significant differences ($p>0.05$) were observed in body weight (BW, Figure 1) or average daily gain (ADG, Figure 2) among the treatment groups during any phase of the study. The dietary inclusion of protease, hydrolyzed tannin or their combinations did not significantly adversely affect BW and ADG over the 42-day trial. Similarly, feed intake (FI, Figure 3) did not differ significantly ($p>0.05$)

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between the control and supplemented groups. However, dietary treatments significantly influenced the cumulative feed conversion ratio (FCR, days 1–42, Figure 4). The HT300 group (300 mg tannin/kg) achieved the highest feed efficient with an FCR of 3.01, which was significantly lower ($p<0.05$) than that of the control group (3.65). The remaining treatment groups (P125, HT150P125, and HT300P125) exhibited intermediate cumulative FCR values ranging from 3.12 to 3.35, which did not differ significantly from either the control or the HT300 group.

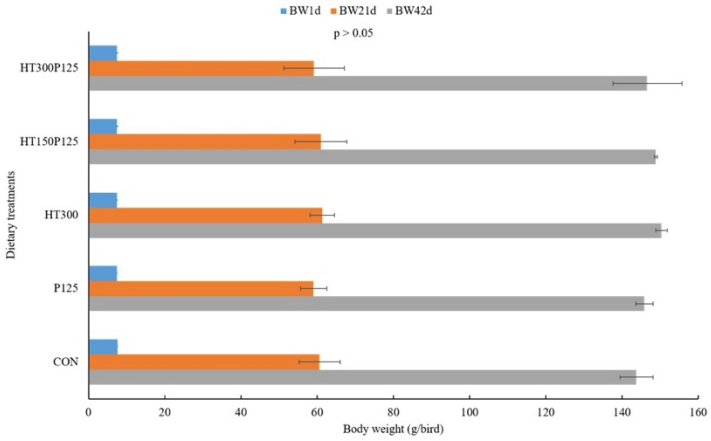


Figure 1 Body weight (BW) of Japanese quail at day 1 (BW1d), day 21 (BW21d), and day 42 (BW42d). Error bars represent the standard deviation (SD). No significant differences were observed among treatments ($p>0.05$). CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg.

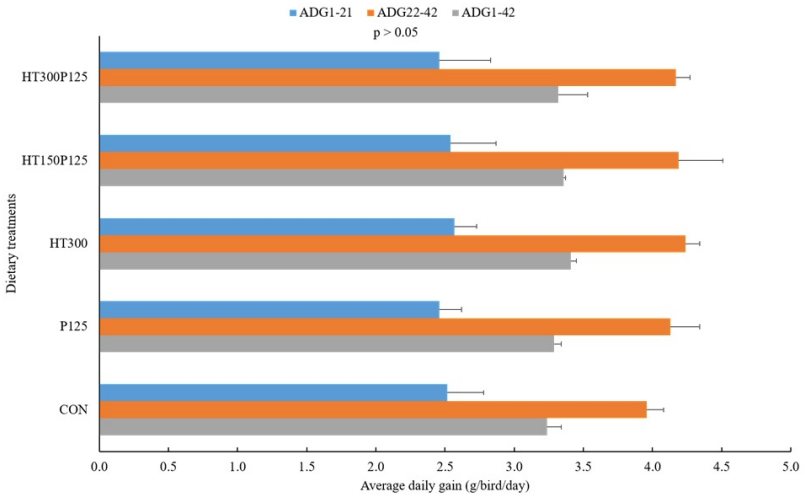


Figure 2 Average daily gain (ADG) of Japanese quail during the starter (ADG1-21), finisher (ADG22-42), and cumulative (ADG1-42) phases. Error bars represent the standard deviation (SD). No significant differences were observed among treatments ($p>0.05$). CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg.

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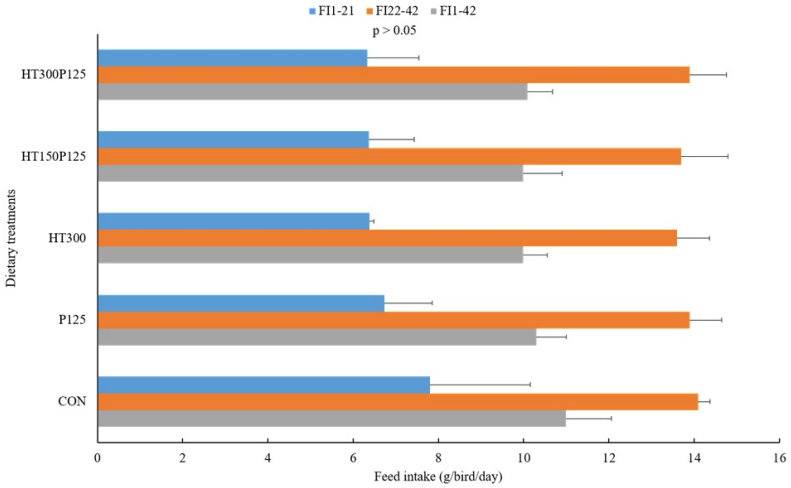


Figure 3 Feed intake (FI) of Japanese quail during the starter (FI1-21), finisher (FI22-42), and cumulative (FI1-42) phases. Error bars represent the standard deviation (SD). No significant differences were observed among treatments (p>0.05). CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg.

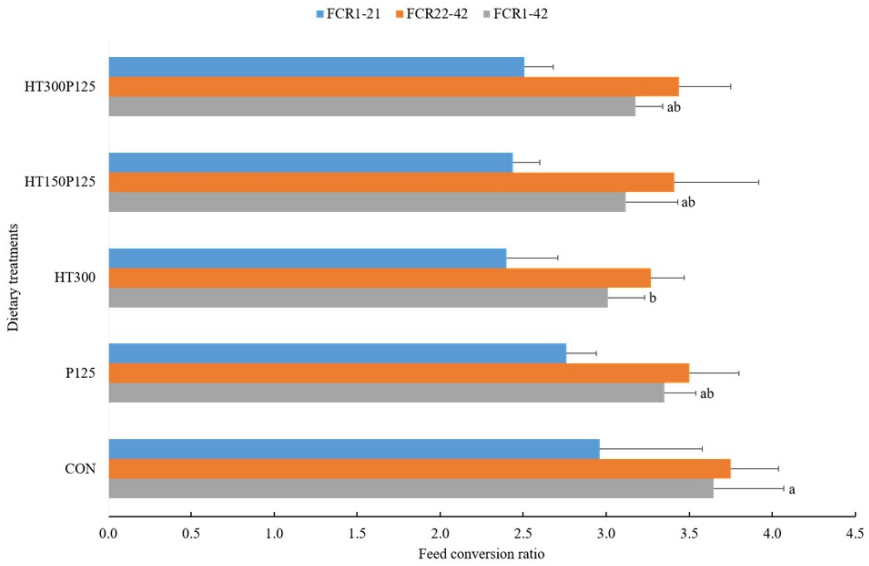


Figure 4 Feed conversion ratio (FCR) of Japanese quail during the starter (FCR1-21), finisher (FCR22-42), and cumulative (FCR1-42) phases. Error bars represent the standard deviation (SD). Different letters (a, b) on the bars within the same phase indicate statistically significant differences among dietary treatments (p<0.05). CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg..

Carcass traits

Dietary treatments did not significantly influence final live weight (LW) or dressing percentage (p>0.05, Table 1). However, the inclusion of dietary additives significantly enhanced breast traits (p=0.05). The highest relative breast percentage (% Breast) and breast meat yield (% Breast meat) were recorded in the HT300P125 group (46.6% and 32.2%, respectively), representing substantial improvements

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over the control group (39.1% and 27.6%, respectively). The remaining supplemented groups (P125, HT300, and HT150P125) displayed intermediate improvements for both parameters. Conversely, no statistically significant differences ($p>0.05$) were observed among the treatment groups regarding the relative weights of commercial cuts (drumsticks and thigh meat) or visceral organs (the total visceral organ mass, gizzard, heart, and liver).

Table 1 Carcass characteristics of the experimental quail

Parameters	Treatment ($\bar{X}$ ± Sd)					P
	CON	P125	HT300	HT150P125	HT300P125	
LW (g/bird)	139.0±12.1	134.9±13.0	148.3±11.7	142.8±10.1	136.5±12.0	0.18
Dressing (%)	70.7±2.51	70.6±1.27	70.73±1.79	69.5±2.33	68.9±2.04	0.28
Breast (%)	39.1±4.00^{b}	42.8±3.10^{ab}	43.2±4.31^{ab}	44.5±5.98^{ab}	46.6±6.28^{a}	0.05
Breast meat (%)	27.6±3.03^{b}	30.7±2.09^{ab}	30.4±3.33^{ab}	30.7±2.70^{ab}	32.2±3.18^{a}	0.05
Drumstick (%)	10.7±0.94	11.2±1.12	10.7±1.12	10.7±0.89	11.0±0.66	0.69
Thigh meat (%)	20.2±1.43	19.6±2.08	20.3±3.21	19.5±2.84	19.2±1.51	0.86
Gizzard (%)	3.23±0.64	3.69±0.43	3.26±0.39	3.22±0.44	3.61±0.48	0.15
Heart (%)	1.27±0.20	1.15±0.13	1.43±0.30	1.36±0.19	1.27±0.28	0.16
Liver (%)	2.75±0.56	3.23±0.34	2.92±0.39	3.08±0.61	3.10±0.50	0.36

a.b Values within a row with different superscripts are significantly different ($p<0.05$). CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg.

Survival rate and oocyst infection rate

Overall, quail survival rates remained high across all treatment groups, ranging from approximately 85.7% to 91.7% (Figure 5). The highest survival rates were observed in the groups supplemented with hydrolyzed tannin alone at 300 mg/kg (HT300) and the low-dose combination group (HT150P125), both exceeding 90%. Conversely, the lowest numerical survival rate was observed in the P125 group (85.7%). However, Chi-square analysis revealed that these numerical variations in survival rates among the control and dietary treatment groups were not statistically significant ($p>0.05$).

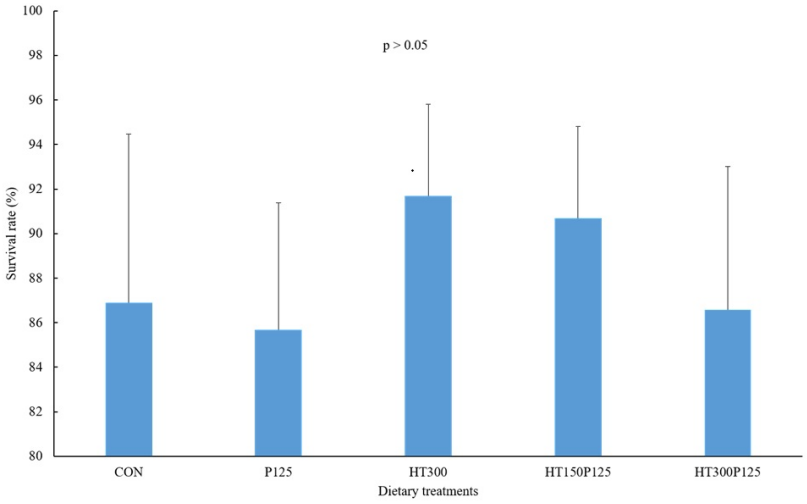


Figure 5 Survival rate (%) of Japanese quail fed diets supplemented with protease and hydrolyzed tannin over a 42-day period. Error bars represent the standard deviation (SD). No significant differences were observed among treatments ($p>0.05$). CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg.

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The percentage of birds testing positive for the presence of coccidial oocysts ranged from 25% to 40% across the treatments (Figure 6). The lowest numerical infection rate was observed in the P125 group (25%), followed by the combination treatment groups HT150P125 and HT300P125, which both exhibited an infection rate of 35%. The highest oocyst detection rates (40%) were observed in the control (CON) and HT300 groups. However, these differences were not statistically significant ($p>0.05$).

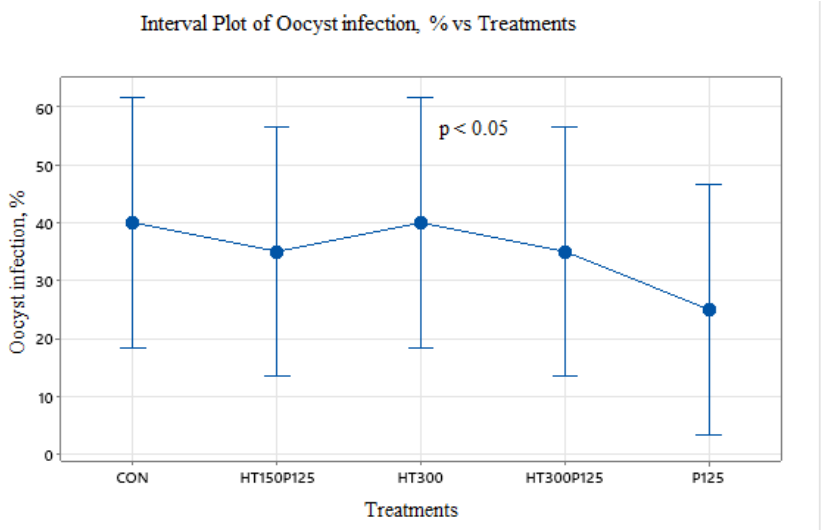


Figure 6 Oocyst infection rate (%) of experimental quail. No significant differences were observed among treatments (Ch-Sq= 0.85; $p>0.05$). The pooled standard deviation is used to calculate the intervals. CON: control; P125: 125 mg protease/kg; HT300: 300 mg tannin/kg; HT150P125: 150 mg tannin/kg + 125 mg protease/kg; HT300P125: 300 mg tannin/kg + 125 mg protease/kg.

DISCUSSION

The experimental quail in this study were fed basal diets formulated with 18-20% CP, which is slightly lower than standard NRC (1994) recommendations for growing Japanese quail. Despite this suboptimal protein level, supplementing the diet with protease and hydrolyzed tannin, either individually or in combination, significantly improved the cumulative FCR and breast meat percentage. These results highlight the efficacy of utilizing exogenous protease and hydrolyzed tannin to optimize nutrient partitioning in protein-restricted quail diets.

The improvements in feed efficiency are primarily attributable to the enzymatic action of exogenous protease, which enhances protein hydrolysis in the gastrointestinal tract, increases amino acid availability. For instance, Ding et al. (2016) found that supplementing diets with 300 mg/kg of protease increased trypsin activity in the pancreas and improved the villus height-to-crypt depth ratio in the intestinal tract. This enhanced nutrient availability likely compensated for the lower dietary CP in the current study. While several studies (Fru-Nji et al., 2011; Park et al., 2020) reported that protease supplementation increased overall BW in poultry, the present study observed improvements strictly in FCR rather than gross BW. This aligns with findings by Freitas et al. (2011) and Ajayi (2015), who observed that exogenous protease supplementation in suboptimal CP diets effectively reduces feed intake and FCR by maximizing the utilization of available amino acids.

The inclusion of hydrolyzed tannins further contributed to these efficiencies, challenging historical perceptions of plant tannins as strictly anti-nutritional factors. The literature on tannin supplementation in poultry reveals highly dose- and source-dependent outcomes. For example, Schiavone et al. (2008) observed that

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supplementing up to 0.20% chestnut hydrolyzed tannins improved daily feed intake and weight gain in broilers. Conversely, Jamroz et al. (2009) reported that while lower doses (0.025% to 0.05%) had no negative effects, a 0.1% inclusion rate reduced final body weight. Furthermore, Marzoni et al. (2020) found that 3% Quebracho tannin (a condensed tannin) severely depressed growth in slow-growing chickens, suggesting that high doses are detrimental. The positive FCR result observed in the current study's HT300 group confirms that a moderate dose of hydrolyzed tannin (300 mg/kg) functions beneficially, avoiding the anti-nutritional threshold while successfully optimizing intestinal nutrient absorption.

Beyond metabolic efficiency, tannins are recognized for their potent antibacterial, antioxidant, and anti-inflammatory properties, which support gut integrity (Scalbert, 1991). Hydrolyzed tannins are particularly noted for their anticoccidial potential, as they form complexes with parasitic enzymes and metal ions crucial for the survival of *Eimeria* spp. (Chung et al., 1998; Min and Hart, 2003). Chestnut tannins has also been shown to decrease secondary pathogen burdens, such as *Clostridium perfringens* in broilers challenged with *Eimeria* species (Tosi et al., 2013). In the context of the present study, these gut-modulating properties likely created a protective intestinal environment, allowing the quail to tolerate subclinical coccidial oocyst loads without compromising systemic health or survival rates.

Ultimately, the combination of protease and tannin in this study yielded a significant synergistic improvement in carcass traits, specifically maximizing relative breast yield in the HT300P125 group. This finding is particularly notable, as it differs from previous research by Fang et al. (2019) and Khang et al. (2022), who reported that protease addition did not affect carcass traits in chickens, as well as Jamroz et al. (2009), who observed no carcass improvements with tannin supplementation. The enhanced breast yield observed here suggests that the exogenous protease successfully liberated critical amino acids from the low-CP basal diet, while the hydrolyzed tannins maintained an optimal, inflammation-free intestinal mucosa, ensuring those amino acids were efficiently absorbed and partitioned toward muscle accretion. Although previous studies reported quail breast percentages between 32.44% and 34.62% (Imad Abdulsahib Al-Shammari, 2023; Khang et al., 2024), our supplemented groups reached values up to 46.6% breast mass, reflecting effective protein deposition under combined additive supplementation.

CONCLUSIONS

The dietary inclusion of exogenous protease and hydrolyzed chestnut tannin proved to be an effective strategy for enhancing the production efficiency of Japanese quail fed diets with slightly suboptimal crude protein levels. Specifically, supplementation with 300 mg/kg of hydrolyzed tannin (HT300) maximized the cumulative feed conversion ratio. Conversely, the co-supplementation of 300 mg/kg hydrolyzed tannin and 125 mg/kg protease (HT300P125) generated a synergistic effect that maximized relative breast meat yield. Therefore, we recommend the inclusion of 300 mg/kg hydrolyzed tannin to optimize feed utilization and maintain host resilience, or the combination of tannin and protease (HT300P125) for producers specifically targeting premium carcass traits.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors declare that Google Gemini was used for language editing—specifically to correct grammar, refine phrasing, and enhance clarity—within this manuscript. The core ideas, arguments, and scientific basis remain the intellectual property of the authors.

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AUTHOR CONTRIBUTIONS

This work was conducted with the contribution of all authors.
Le Thanh Phuong: Conceptualization (lead); Writing – original draft (lead); Formal analysis; Writing – review and editing (equal).
Tran Thi Thuy Hang: Conceptualization (supporting); Data curation (supporting); Writing – review and editing (equal).
Nguyen Thi Huynh Anh: Visualization (lead); Methodology (lead); Data curation (lead); Writing – review and editing (equal).
Nguyen Ho Bao Tran: Visualization (lead); Methodology (supporting); Writing – review and editing (equal).
Nguyen Thi Kim Khang: Conceptualization (supporting); Supervision (lead); Writing – review and editing (equal).

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