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# Kasturi lime (*Citrofortunella microcarpa*) waste in quail diet: Effect on early laying performance and egg quality

Rina Muryani*, Edjeng Suprijatna, Binti Ma'rifah, Luthfi Djauhari M, Dwi Sunarti, Hanna Dzawish Shihah

*Department of Animal Science, Faculty of Animal and Agricultural Sciences Universitas Diponegoro, Semarang 50275, Central Java, Indonesia.*

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*Correspondence:

Corresponding author: Rina Muryani
E-mail address: rinamuryani@lecturer.undip.ac.id

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**ABSTRACT**

Japanese quails (*Coturnix coturnix japonica*) are efficient egg producers, laying 200–300 eggs annually from 42 days of age. However, their productivity and egg quality are often compromised by Indonesia's hot tropical climate. Kasturi lime (*Citrofortunella microcarpa*) waste from local beverage industries still contains bioactive compounds which may help mitigate these stressors. This study aimed to evaluate the effect of kasturi lime waste powder (KLWP) in the diets of Japanese quails in the early laying phase on their production performance and egg quality. A total of 280 six-week-old female quails were assigned to four dietary treatments: 0% (K0), 2% (K1), 4% (K2), and 6% (K3) KLWP, with seven replicates of ten birds per treatment, and housed in tiered cages for 56 days. The average temperature in the cage was 28-32°C, with a relative humidity of 65-81% and a Heat Stress Index of 95±7.8. There were no differences between the treatments in terms of laying rate, feed conversion ratio, eggshell weight, and yolk index. However, a linear regression was obtained for feed intake, and a quadratic response was observed for egg mass and egg weight. After five days of storage, eggs from the KLWP group retained more weight than those from the control group. KLWP supplementation, especially at 2%, enhanced egg mass and quality without compromising bird health, supporting its potential as a sustainable feed additive.

## Introduction

Layer quail farming plays an essential role in animal protein production, particularly through the consistent supply of eggs. Japanese layer quails (*Coturnix coturnix japonica*) are small, highly productive, and economically viable poultry species that begin laying eggs at approximately six-weeks of age, with an annual output of 200–300 eggs (Lobato *et al*., 2025). They perform optimally at temperatures between 24 and 30°C and relative humidity levels of 60–70%. However, layer quails are highly sensitive to stress, especially heat stress, which can impair feed intake, egg production, and overall egg quality (Khang *et al*., 2024). These challenges are more pronounced in tropical and arid climates. High-fiber diets may exacerbate the issue, as elevated temperatures accelerate the maturity of plant-based feed ingredients. Antioxidants have been shown to reduce stress and improve performance in laying quails (Kusumasari *et al*., 2013).

Meanwhile, the food industry, especially the fruit beverage sector, is facing increasing environmental challenges due to industrial growth and rising waste generation. In Indonesia alone, food industry waste ranges from 23 to 48 million tons annually, equating to 115–184 kg per capita per year (Kemenperin, 2021). Globally, nearly 50% of fruit and vegetable production is being wasted. Among these, Kasturi lime (*Citrofortunella microcarpa*), also known as calamondin or calamansi, is a significant byproduct of the Citrofortunella processing industry in Southeast Asia, including Indonesia, the Philippines, Vietnam, China, Singapore, and Malaysia (Zou *et al*., 2020; Venkatachalam *et al*., 2023). Kasturi lime is a hybrid citrus species derived from a cross between Citrus reticulata Blanco (mandarin) and Fortunella sp. (kumquat) (Lou and Ho, 2017). The fruit is small (1.5–3 cm in diameter), round, fragrant, and has a thin green skin that turns bright orange upon ripening. It is widely used for juice extraction and culinary applications, particularly to eliminate fishy odor from seafood dishes.

Kasturi lime waste contains a diverse array of bioactive compounds, including flavonoids, pectin, essential oils, sugars, and limonin (Zou *et al*., 2020; Venkatachalam *et al*., 2023). The pectin content ranges from 8.14% to 15.3% (Rosalina *et al*., 2017), underscoring its potential as a functional feed ingredient. Citrus pomace, the byproduct remaining after juice extraction, comprises peel, pulp, and membrane tissues, and is rich in essential oils, organic acids, carotenoids, dietary fiber, polyphenols (flavonoids and non-flavonoids), vitamins (B2, B3, B6, and E), and trace minerals such as calcium and potassium (Rafiq *et al*., 2018). The flavonoid profile of Kasturi lime pomace includes diosmin, hesperetin, hesperidin, naringin, and quercetin, which are known for their antioxidant and antimicrobial properties (Zou *et al*., 2020). Given its bioactive properties and local abundance, Kasturi lime waste presents a novel opportunity to enhance layer quail performance under heat stress while promoting sustainable waste management. This study aimed to evaluate the effects of Kasturi lime waste powder (KLWP) on early egg production and quality in Japanese layer quails.

## Materials and methods

### *Experimental design and treatments*

A total of 280, 6-week-old female quails (average initial weight: 139.88±14.10 g) were obtained from a local farm and reared under controlled conditions. The study was a Completely Randomized Design (CRD) with four dietary treatments: control (0% KLWP, K0), 2% KLWP (K1), 4% KLWP (K2), and 6% KLWP (K3). Each treatment had seven replicates, with 10 birds per replicate.

### *Feed preparation*

Kasturi lime (*Citrofortunella microcarpa*) waste was sourced from a juice manufacturing company in Bekasi, Indonesia. After seed removal, the peel and pulp were sun-dried under natural sunlight for approximately 4 h (06:00–09:00, in 21-28°C) to reduce surface moisture and promote aeration. The materials were then dehydrated using a vertical air-flow dryer at 35°C for two days until the moisture content fell below 10%. The dried waste was ground into a fine powder using a hammer mill.

Four dietary treatments were formulated to be isocaloric and isonitrogenous, based on NRC (1994) standards, to meet the nutritional requirements of Japanese layer quails. The proximate composition of the

Kasturi lime powder was determined using AOAC (1990) methods (Table 1), and the feed formulations were analyzed accordingly (Table 2). The metabolizable energy (ME) of the diets was calculated using the indirect method: ME (Kcal/kg DM)=3951+54.4EE−88.7CF−40.8 (Wiseman, 1987). The chemical composition of Kasturi lime powder is presented in Table 1, and the composition and nutritional content of the quail feed are listed in Table 2.

Table 1. Chemical composition of Kasturi lime waste powder.

| Nutritional contents | Composition |
|---|---|
| Dry matter (%)[1] | 89.29 |
| Crude protein (%)[1] | 7.17 |
| Crude Fiber (%)[1] | 8.08 |
| Crude Fat (%)[1] | 1.59 |
| Ash (%)[1] | 7.54 |
| Energi Metabolic (kcal)[2] | 2936 |
| Nitrogen Free Extract (%)[3] | 65.91 |
| Total phenolic content (mg GAE / g DW)[2] | 12.74 |
| Pectin content (%)[4] | 8.2 |
| Vitamin C (mg/g)[3] | 17.6 |
| Antioxidant activity [3] | 19.92 |

[1]Proximate anaysis; [2]ME calculation (Wiseman, 1987); [3]DPPH

Table 2. Composition and nutritional content of experimental diets.

| Feedstuff | K0 | K1 | K2 | K3 |
|---|---|---|---|---|
| Corn | 53 | 52 | 50.7 | 48 |
| Soybean meal | 26.9 | 26.9 | 26.9 | 26.9 |
| Meat Bone Meal | 8 | 8 | 8 | 8 |
| Fish Meal | 7.7 | 7.7 | 7.7 | 7.7 |
| Rice bran | 2 | 1 | 1 | 1 |
| Palm Oil | 0.5 | 0.5 | 0.5 | 0.5 |
| Premix[1] | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt | 0.08 | 0.08 | 0.08 | 0.08 |
| L-Lysin | 0.47 | 0.47 | 0.47 | 0.47 |
| Limestone | 0.85 | 0.85 | 0.85 | 0.85 |
| Kasturi Lime Waste Powder (KLWP) | 0 | 2 | 4 | 6 |
| Dry Matter (%) | 89.62 | 89.59 | 89.64 | 89.66 |
| Crude Protein (%) | 20.75 | 20.57 | 20.38 | 20.2 |
| Ether extract (%) | 5.63 | 5.55 | 5.5 | 5.46 |
| Crude Fiber (%) | 8.25 | 8.15 | 8.11 | 8.07 |
| Ash (%) | 8.91 | 8.26 | 9.04 | 9.16 |
| Nitrogen Free Extract (%) | 46.08 | 47.11 | 46.61 | 46.77 |
| Metabolizable Energy (kcal/kg) | 3155 | 3163 | 3159 | 3155 |
| Calcium (%) | 1.02 | 1.06 | 1.17 | 1.1 |
| Phosphor available (%) | 0.45 | 0.5 | 0.58 | 0.55 |
| Pectin content (g/kg) | - | 1.64 | 3.28 | 6.56 |

Note: K0 = Control Diet, K1=Diet 2%KLWP, K2=Diet 4%KLWP, K3= Diet 6% KLWP.
[1].Premix contained (per kg) of vitamin A 7,750 IU, vitamin D 1,550 IU, vitamin E 1,88 mg, vitamin B1 1.25 mg,vitamin B2 3.13 mg, vitamin B6 1.88 mg, vitamin B12 0.01 mg, vitamin C 25 mg, folic acid 1.50 mg, Ca-d-pantothenat 7.5 mg, niacin 1.88 mg, biotin 0.13 mg, BHT 25 mg

## Results

The production performance of laying quails fed different dietary regimes is shown in Table 3. KLWP supplementation significantly influenced feed intake, egg mass, and egg weight ($p < 0.05$) over 56 days at 14 weeks old. The K1 group (2% KLWP) showed higher feed intake than control (K0), with no differences between K2 (4%) and K3 (6%) groups ($p > 0.05$). Egg mass was highest in K1 compared to K0, with no differences between K2 and K3. Egg weight in K3 exceeded K0 and K2 but matched K1. Laying rate and feed conversion ratio remained unchanged across treatments.

Digestive organ weights varied with diet (Table 4). Crop weight decreased with higher KLWP, while proventriculus and small intestine weights increased ($p < 0.05$). Ventriculus, cecum, and colon weights were unaffected ($p > 0.05$). KLWP affected protein and ash digestibility ($p < 0.05$), but not crude fiber digestibility (Table 5). K2 showed lowest protein digestibility, while K2 and K3 showed increased ash digestibility.

Table 6 shows egg quality parameters. KLWP affected eggshell thickness and Haugh units ($p < 0.05$), but not shell weight or yolk index. Eggshell thickness increased in K1, K2, and K3 compared to K0. Haugh unit was highest in K2. After 5d storage, K3 showed highest egg weight and Haugh unit, though similar to K1 and K2. Shell weight and yolk index remained unchanged.

## Discussion

Kasturi Lime Waste Powder (KLWP), from Citrofortunella macrocarpa pomace, contains 7.17% protein, 8.08% crude fiber, 1.59% crude fat, 17.6 mg/g vitamin C, 12.74 mg/g GAE total phenolics, and 8.20% pectin, with antioxidant activity (IC50) of 19.92 μg/mL (Table 1). Compared to general citrus waste (Saeed *et al*., 2024) with 7% protein, 14% fiber, and 2.5% fat, KLWP has similar protein but lower fiber and fat. Wang *et al*. (2007) found Kasturi lime waste contains bioflavonoids like diosmin and quercetin, contributing to its antioxidant capacity. Lemon peel powder in laying quail diets had 53.31% crude fiber, 4.36% fat, 14.60 mg/g GAE phenolics, and antioxidant capacity (IC50 of 5.58 μg/mL). These comparisons indicate lemon peel has higher fiber and antioxidant activity, while KLWP provides a balanced nutrient profile for quail feed.

Table 3 presents laying quails' production performance under different dietary regimes. Feed intake increased, correlating with improved egg mass without affecting laying rate and feed conversion ratio. The enhanced egg mass and weight at 14 weeks can be attributed to KLWP phytochemicals, including dietary fiber, polyphenols, organic acids, and essential oils (Zou *et al*., 2020; Venkatachalam *et al*., 2023), which support metabolic activity and quail health. Florou-Paneri *et al*. (2001) reported that 6% dried sweet orange pulp could be included in laying quails' diet without impairing performance. Nazok *et al*. (2010) showed that 12% dried citrus pulp in laying hen diets had no impact on feed intake or FCR. However, Goliomytis *et al*. (2018) found that 9% sweet orange pulp decreased feed intake and laying rate while increasing FCR in laying hens.

Increased feed intake with citrus by-products has been documented in broiler chickens at various inclusion levels: 2% (Abbasi *et al*., 2015), 5% (Mourao *et al*., 2008). Both broilers and quails increase feed intake during growth and early egg-laying phases. D'Avila Lima *et al*. (2015) ob-

Table 3. The production performance of 14-weeks-old quail.

| | Kasturi Lime Waste Powder (KLWP) | | | | SEM | p-value | P | | |
|---|---|---|---|---|---|---|---|---|---|
| | K0 | K1 | K2 | K3 | | | Linier | Quadratic | Cubic |
| Feed intake, g/d | 20.38[b] | 21.58[a] | 21.57[a] | 22.05[a] | 0.81 | 0.01 | 0.00 | 0.27 | 0.24 |
| Laying rate, % | 73.26 | 76.78 | 70.79 | 73.45 | 3.86 | 0.15 | 0.93 | 0.02 | 0.98 |
| Egg mass, g/bird/d | 7.25[b] | 7.85[a] | 7.23[ab] | 7.66[ab] | 0.48 | 0.03 | 0.09 | 0.03 | 0.22 |
| FCR for egg | 2.82 | 2.75 | 2.8 | 2.88 | 0.14 | 0.47 | 0.36 | 0.22 | 0.70 |

[abc] different superscripts on the same row indicate significant different(P<0.05)

Table 4. Digestive organs growth.

| | KLWP, % | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | K0 | K1 | K2 | K3 | | |
| Crop, % | 0.47$^{a}$ | 0.38$^{b}$ | 0.39$^{b}$ | 0.37$^{b}$ | 0.06 | 0.03 |
| Proventriculus, % | 0.44$^{b}$ | 0.45$^{b}$ | 0.46$^{ab}$ | 0.52$^{a}$ | 0.05 | 0.02 |
| Ventriculus, % | 2.19 | 2.07 | 1.99 | 2.3 | 0.35 | 0.44 |
| Small intestine, % | 2.87$^{b}$ | 2.79$^{b}$ | 2.94$^{b}$ | 3.23$^{a}$ | 0.27 | 0.04 |
| Secum, % | 0.66 | 0.62 | 0.63 | 0.71 | 0.29 | 0.35 |
| Colon, % | 0.3 | 0.25 | 0.26 | 0.36 | 0.18 | 0.46 |
| Liver, % | 2.51 | 2.57 | 2.45 | 2.54 | 0.23 | 0.62 |

$^{abc}$ different superscripts on the same row indicate significant different(P<0.05)

Table 5. Nutrient digestibility and retention.

| | KLWP, % | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | K0 | K1 | K2 | K3 | | |
| Nutrient digestibility | | | | | | |
| Protein, % | 75.50±3.24$^{a}$ | 72.34±4.61$^{ab}$ | 66.41±3.92$^{b}$ | 70.84±3.44$^{ab}$ | 3.80 | 0.04 |
| Ash, % | 20.07±2.41$^{b}$ | 27.69±2.22$^{b}$ | 28.12±2.81$^{ab}$ | 37.03±3.33$^{a}$ | 2.69 | 0.03 |
| Crude fibre, % | 34.69±4.28 | 34.44±3.84 | 35.34±2.90 | 36.73±2.41 | 3.36 | 0.68 |
| Nutrient retention | | | | | | |
| Protein, g | 3.21±0.16 | 3.20±0.29 | 3.12±0.12 | 3.25±0.16 | 0.18 | 0.21 |
| Ash, g | 0.35±0.01$^{b}$ | 0.51±0.02$^{b}$ | 0.55±0.03$^{ab}$ | 0.69±0.03$^{a}$ | 0.02 | 0.03 |

$^{abc}$ different superscripts on the same row indicate significant different(P<0.05)

Table 6. Egg quality.

| | KLWP, % | | | | SEM | p-value |
|---|---|---|---|---|---|---|
| | K0 | K1 | K2 | K3 | | |
| Egg weight, g | 9.90$^{b}$ | 10.24$^{ab}$ | 10.06$^{b}$ | 10.44$^{a}$ | 0.25 | 0.00 |
| Shell weight, g | 0.84 | 0.89 | 0.87 | 0.88 | 0.06 | 0.46 |
| Shell thickness, mm | 0.17$^{b}$ | 0.20$^{a}$ | 0.19$^{a}$ | 0.19$^{a}$ | 0.01 | 0.01 |
| Haugh unit | 97.68$^{b}$ | 99.40$^{ab}$ | 105.95$^{a}$ | 103.87$^{a}$ | 3.52 | 0.01 |
| Yolk index | 0.83 | 0.85 | 0.86 | 0.86 | 0.07 | 0.17 |
| After 5 days in storage | | | | | | |
| Egg weight, g | 9.31$^{c}$ | 9.46$^{b}$ | 9.45$^{bc}$ | 10.08$^{a}$ | 0.30 | 0.03 |
| Haugh unit | 96.36$^{b}$ | 98.04$^{a}$ | 94.95$^{b}$ | 95.19$^{b}$ | 2.76 | 0.03 |
| Yolk index | 0.57 | 0.55 | 0.52 | 0.46 | 0.08 | 0.55 |

$^{abc}$ different superscripts on the same row indicate significant different(P<0.05)

served that feed intake in Japanese quails increased during growing and early production phases, with optimal body weight being crucial for egg production. Although fermentable sugars were not directly quantified in this study, citrus pomace is known to contain them (Mnisi *et al*., 2022), along with dietary fibers and organic acids, which collectively enhance gut health and nutrient absorption. In this study, quails likely consumed more feed not only to satisfy their energy requirements but also because of the health benefits derived from KLWP inclusion. Fermentable sugars and dietary fibers in citrus waste serve as prebiotic substrates for beneficial gut microbiota, stimulating microbial fermentation and the production of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. These SCFAs play a crucial role in lowering intestinal pH, strengthening the gut lining, and enhancing mineral absorption, thereby promoting digestive health and microbial balance (Singh and Kim, 2021, Jha and Mishra, 2021). Citrus by-products also contain organic acids, such as citric, acetic, and oxalic acids, which exhibit antimicrobial properties that suppress pathogenic bacteria and fungi. Additionally, these acids enhance fiber functionality by slowing starch digestion and stabilizing the postprandial blood glucose levels. The synergistic action of fibers, organic acids, and polyphenols in KLWP supports a healthier gut environment, improves nutrient utilization, and helps maintain a stable microbial community (Jha and Mishra, 2021).

KLWP's rich content of polyphenols, along with other secondary metabolites commonly found in citrus waste, such as flavonoids and carotenoids, contributes significantly to its beneficial effects. These compounds possess potent antimicrobial, anti-inflammatory, and antioxidant properties that further support gut health and systemic well-being. Zou *et al*. (2020) specifically described Kasturi lime (Citrofortunella macrocarpa) waste as rich in citric, dehydroascorbic, and ascorbic acids (vitamin C), as well as polyphenolic acids, flavonoids (e.g., diosmin, hesperidin, naringin, quercetin), and essential oils (e.g., d-limonene). These compounds contribute to antioxidant and antimicrobial activities. These compounds may also mitigate oxidative stress and enhance appetite during the early laying periods (Mnisi *et al*., 2022). Indeed, consistent with the multifaceted benefits of these compounds, feed intake exhibited a linear increase with KLWP inclusion, while egg mass followed a quadratic response pattern (Table 3).

Nutritional effects are often primarily assessed via feed intake. Richard and Proszkowiec-Weglarz (2007) further stressed that feed intake and energy expenditure must be tightly regulated to maintain energy balance and body weight. In quails, satiety is predominantly influenced by crop fill limitations and the fulfillment of energy requirements (Batool *et al*., 2021; Classen *et al*., 2016). This aligns with our observed physiological changes, such as reduced crop weight and increased proventriculus and small intestine weights (Table 4) due to KLWP inclusion, suggesting enhanced digestive tract activity to meet nutritional demands.

The observed increase in daily feed intake across KLWP inclusion levels (21.38–22.05 g/bird/day) remained within acceptable ranges when compared with previous reports. For instance, Edi and Andri (2023) recorded 23.72 g/bird/day at 2900 kcal/kg metabolizable energy (ME) and 21% protein Despite the higher dietary energy (3100 kcal/kg ME) and crude fiber content (8%) used in the present study, the feed intake did not decline. This suggests that laying quails possess a high tolerance and digestive adaptability to fibrous diets, possibly supported by their relatively larger cecum and more developed intestinal mucosa. In contrast to studies reporting reduced feed intake in laying hens due to citrus polyphenols and dried citrus pulp (Goliomytis *et al*., 2018), quails showed no reduction with KLWP inclusion up to 6%; instead, feed consumption increased. Quails generally tolerate citrus waste in feed at levels up to 12% without adverse effects on intake (Florou-Paneri *et al*., 2001). Unlike these extracts, this study used KLWP in powdered form, retaining a broader nutritional matrix, including fiber, sugars, and essential oils, which may moderate bitterness. Powdered herbal feed additives enhance palatability, though effects vary by fiber type and level (Kholiavska *et al*., 2025). Roura *et al*. (2013) reported that quails have 62 taste buds, fewer than Rhode Island Red hens (256), limiting taste sensitivity. While quails can detect bitter compounds, they prefer sweet flavors, influencing their feed intake and dietary selection.

The inclusion of up to 6% KLWP (K3) in quail feed did not negatively affect laying rate or feed conversion but increased egg mass. Similarly, Nazok *et al*. (2010) found that up to 12% dried citrus pulp in laying hen feed did not affect laying rate or feed conversion, though it influenced egg mass at ≥ 8% inclusion. Lemon peel powder (up to 0.5%) in laying quail feed did not affect laying rate or performance parameters. Unlike KLWP, neither dried citrus pulp nor lemon peel powder increased feed intake or improved feed conversion at moderate inclusion levels (Nazok *et al*., 2010).

The polyphenol intakes in the K1, K2, and K3 diet groups were 5.45±0.22, 10.85±0.53, and 16.41±0.57 mg/bird/day, respectively. These values correspond to dietary concentrations of 255 mg/kg (K1), 510 mg/kg (K2), and 765 mg/kg ( 0.765 g/kg) of citrus polyphenols in the feed. They were sufficient to maintain laying rate and increase egg mass in quails. Polyphenols are well known to enhance antioxidant status and gut health owing to their antimicrobial properties, thereby supporting the overall health and reproductive performance of quails. For instance, polyphenols, such as hesperidin and naringenin, when included at levels up to 1 g/kg in layer diets improved egg production.

Polyphenols exhibit antimicrobial and antioxidant properties. They help balance the gut microbiota (Abdel-Moneim *et al*., 2020), enhance antioxidant capacity at the cellular level, reduce oxidative damage, and improve the overall health. These actions collectively support improved egg production (Yuan *et al*., 2016; Liu *et al*., 2014). For example, Yuan *et al*. (2016) found that laying hens fed 0.5–0.6 g/kg green tea extract showed improved feed efficiency and increased egg production. Additionally, 5 and 10 g/kg turmeric (Radwan *et al*., 2008) and 0.150 g/kg curcumin (Liu *et al*., 2020) have been shown to improve the productivity of laying hens. Although feed intake increased, protein retention remained unchanged, likely due to decreased protein digestibility (Table 2). This decrease may be attributed to the presence of anti-nutritional factors (ANFs) in KLWP, which can inhibit protein digestion. Citrus pomace, including KLWP, may contain various ANFs, such as condensed tannins, trypsin inhibitors, oxalates, phytates, and non-starch polysaccharides (e.g., pectin, cellulose, hemicellulose, beta-glucans, and xylans) (Mnisi *et al*., 2022). High levels of soluble fibers, such as pectin and polyphenols, can also inhibit digestive enzymes. Pectin and other dietary fibers can physically and chemically reduce enzyme activity, affecting nutrient availability for egg formation. Specifically, pectin may interfere with nutrient absorption by increasing digestive tract viscosity (Mnisi *et al*. 2022). Similarly, high concentrations of polyphenols may impair nutrient digestibility in quails by inhibiting digestive enzymes and binding to bile salts, thereby reducing protein and fat absorption (Abdel-Moneim *et al*., 2020)

The inclusion of KLWP in the diet significantly elevated vitamin C concentration, with diets K1, K2, and K3 providing supplementary vitamin C from KLWP at 352, 704, and 1056 mg/kg, respectively. These findings align with those of Sigolo *et al*. (2019), who reported that maximum feed intake and laying rate in laying quails were achieved with dietary vitamin C levels of 800 or 1000 mg/kg over a 42-63 day period. Similarly, Sahin *et al*. (2009) and Abdulameer (2019) found that supplementing broiler diets with 500 mg/kg vitamin C increased feed intake; interestingly, they noted that this was comparable to 1% dried sweet orange peel inclusion, and that increasing sweet orange peel to 2% resulted in the highest feed intake. Conversely, Asensio *et al*. (2020) observed that a lower inclusion rate of 200 mg/kg vitamin C did not affect performance but reduced the incidence of chickens pecking non-food objects, likely due to stress mitigation.

In t/he present study, the overall egg production was slightly lower than the generally reported standard for this age group. The low production observed specifically in the control group (K0, without KLWP inclusion) is likely due to its high fiber content, which can reduce net energy consumption and consequently affect production. A high fiber component in the feed reduces the available energy because its digestion requires significant metabolic energy, leaving quails with less energy for productive purposes. Salombre *et al*. (2018) similarly noted that digesting fiber demands more energy, often leading to insufficient energy reserves for production in quails.

Table 3 illustrates the variations in the weights of several organs within the digestive tract, reflecting physiological adaptation to the experimental dietary regimen. Specifically, the relative weight of the crop decreased with increasing KLWP levels, whereas the weights of the proventriculus and small intestine tended to increase ($p < 0.05$). In contrast, the ventriculus (gizzard), cecum, and colon were not significantly affected by the treatment ($p > 0.05$). These observed changes in organ mass can be attributed to the synergistic effects of organic acids, polyphenols, essential oils, dietary fiber, and other bioactive compounds present in KLWP. Collectively, these components enhanced the overall mechanisms of digestion and absorption within these specific organs.

In the crop, feed or ingesta accumulate, becoming moist and softened, which then initiates the activity of feed-derived, endogenous, exogenous, and microbial enzymes (Classen *et al*., 2016). The soluble dietary fiber in KLWP-containing diets likely accelerated ingesta movement from the crop to the proventriculus in quails. Specifically, the increased viscosity induced by citrus pectin, combined with the limited crop capacity in quails, may accelerate crop emptying as the ingesta becomes more fluid-like and transitions swiftly to the proventriculus. This mechanism consequently led to the observed decrease in crop weight with increasing KLWP inclusion in the diet. As a storage organ, the crop emptying rate is significantly influenced by the physical properties of the ingesta and gizzard activity.

Longer retention of ingesta may occur in the proventriculus and small intestine, thereby enhancing the digestion process, particularly because of the presence of organic acids, essential oils, polyphenols, and soluble fibers in KLWP. The proventriculus, ventriculus (gizzard), and intestines, which are essential for digestion and nutrient absorption, are significantly responsive to changes in dietary composition (Tejeda and Kim, 2021). Basir and Toghyani (2017) reported that supplementing broiler diets with 2.5%-12% dried lemon pulp increased the weight of all parts of the small intestine, including the duodenum, jejunum, and ileum.

Organic acids, such as citric acid, can facilitate a reduction in gut pH, thereby improving the digestibility of protein and crude fiber. Supplementation with various organic acids, including citric acid, acetic acid and especially ascorbic acid has been shown to significantly increase the height and width of the small intestinal villi. Ascorbic acid particularly influences gut morphology by increasing gut tensile strength and improving epithelial morphology, including enhancing villus height and reducing epithelial thickness. These favorable changes ultimately enhance nutrient absorption and improve overall gut health. Furthermore, ascorbic acid can interact with other dietary components, such as probiotics, to improve gut integrity and immune responses (Ognik *et al*., 2019).

Limonene, the primary essential oil in citrus lime waste, indirectly promotes enzyme secretion (Eratak *et al*., 2023). Similarly, polyphenols, such as naringin, naringenin, and hesperidin, support a healthy digestive tract through their antioxidant and antimicrobial properties (Abdel-Moneim, 2020). In addition, various components of dietary fiber can modulate the structure and functionality of the gastrointestinal tract, thereby affecting nutrient metabolism and performance (Tejeda and Kim, 2020 ; Jha and Mishra, 2021). These findings indicate that variations in gut size are predominantly associated with feed intake rather than solely the direct effects of dietary fiber, suggesting that quails modulate feed consumption to meet specific dietary needs beyond their energy requirements.

In this study, the inclusion of KLWP did not improve protein digestibility; instead, it tended to decrease it (Table 4). However, it notably enhanced the digestibility of ash. Protein digestibility showed no significant changes up to 2% KLWP (K1); however, it declined significantly at 4% (K2), then increased again at 6% (K3), returning to levels similar to those of the 0% (K0) and 2% (K1) inclusions. At the 4% KLWP inclusion level (K2), pectin and other fibers inhibited protein digestive enzymes, and the beneficial contributions of organic acids and polyphenols were insufficient to counteract this effect. Pectin interferes with the activity of digestive enzymes such as amylase, chymotrypsin, and trypsin. Conversely, at 6% (K3) inclusion, the beneficial effects of accumulated citric acid and polyphenols likely overcame these inhibitory effects, appearing to restore feed protein digestibility. Similarly, ash digestibility (reflecting mineral content) showed no significant difference between the control group (K0) and the 2% KLWP inclusion group (K1). However, it notably increased at the 4% KLWP inclusion level (K2) and further increased at the 6% KLWP inclusion level (K3). The combined presence of dietary fiber, polyphenols, and organic acids in KLWP positively affects mineral digestion and absorption. Previous research has confirmed that organic acids can enhance the digestibility of minerals by creating a favorable gut environment for nutrient absorption. Additionally, the increased ingesta retention time attributed to citrus pectin, coupled with the presence of organic acids and polyphenols in the digestive tract, could enhance mineral solubility and absorption (Table 4). This is because acidic anions from these compounds can form soluble complexes with essential minerals such as Ca, phosphorus, Mg, and Zn, thereby improving their digestibility

The extended retention of ingesta within the proventriculus and small intestine, enhanced by various phytochemicals from KLWP, likely leads to improved digestive efficiency. This prolonged retention time may also encourage organ enlargement as an adaptation, consistent with the observed increase in proventriculus and small intestine weights at higher KLWP inclusion levels (Table 3). Furthermore, polyphenols are known to enhance mineral uptake through their antimicrobial and antioxidant properties, which promote gut health. Their ability to chelate metal ions can increase mineral bioavailability and prevent the formation of insoluble complexes, contributing to enhanced mineral absorption.

Organic acids improve growth performance by lowering gut pH, inhibiting pathogenic bacteria, and enhancing nutrient utilization, making them effective substitutes for antibiotic growth promoters. They also enhance gut health by modulating microbiota, reducing inflammation, and fortifying intestinal barrier integrity, while simultaneously increasing SCFA production, which contributes to anti-inflammatory properties. According to Attia *et al*. (2018), dietary organic acids can specifically promote the development of beneficial microbiota such as Lactobacillus spp. Citric acid, for instance, increases the number of beneficial bacteria in the ileum and cecum, helping to balance gut flora and improve feed utilization. Overall, the beneficial balance of microbiota in the digestive tract is strongly supported by the collective actions of organic acids, polyphenols, and essential oils from citrus waste.

Soluble fibers can retain water and form gels, thereby slowing the passage of digesta through the gastrointestinal tract. This process, in turn, can improve the duodenal mucosa and cecal villus-to-crypt ratio. Importantly, dietary fibers can interact with polyphenols in the digestive tract, forming associations that affect their bioaccessibility and absorption. Dietary fibers tend to lower the amount of polyphenols released in the upper digestive tract, potentially increasing the amount that reaches the lower parts of the digestive tract, where they can exert beneficial effects. Additionally, soluble fiber is easily fermentable, supporting microbiota balance due to increased viscosity and retention time of the ingesta. This enhanced microbiota activity also plays a crucial role in improving the availability of polyphenols for absorption in the small intestine and colon, as microbial enzymes convert less available glycoside forms into more absorbable aglycone forms (Abdel-Moneim *et al*., 2020). Therefore, utilizing the correct amount of KLWP, which provides an optimal composition of soluble fiber, polyphenols, and organic acids, offers greater holistic benefits than administering these components separately

In this study, the ventriculus (gizzard) displayed no substantial changes (Table 3). This can be attributed to the fact that KLWP inclusion did not significantly alter the ratio of insoluble to soluble dietary fiber, nor was there a significant variation in particle size across the diets. Both fiber type and particle size are critical variables for stimulating muscular activity in the ventriculus and, consequently, its size (Tejeda and Kim, 2020). Although KLWP inclusion reduced the proportion of alternative feed ingredients, such as corn and rice bran (primary sources of insoluble fiber), the overall crude fiber percentage in the diets (8%) remained consistent. While previous studies show that both soluble and insoluble dietary fibers significantly affect gut health, intestinal morphology, digestive organ development, and nutrient absorption in quails (Rezaei *et al*., 2018), the lack of effect on the cecum and colon in our study indicates no significant alteration in overall fiber digestibility of the diet, as shown in Table 4.

The egg quality of laying quails fed different dietary regimes is shown in Table 6. The inclusion of KLWP in quail rations significantly affected egg weight, eggshell thickness and Haugh unit ($p<0.05$) over a 56-day period. No significant differences were observed in eggshell weight or yolk index among the groups. Eggshell thickness increased significantly in the K1 group with 2% KLWP compared to that in the control group, K0. K2 (4% KLWP) and K3 (6% KLWP) also showed higher eggshell thicknesses. The Haugh unit was higher in the K2 group (4% KLWP) than in the control group (K0). The K1 (2% KLWP) and K3 (6% KLWP) groups also showed improved Haugh unit values. No significant differences were observed in the yolk index among the groups. The inclusion of kasturi lime waste powder (KLWP) in the diet of laying quails significantly influenced several egg quality parameters, particularly egg weight, eggshell thickness, and Haugh unit (HU). These improvements are consistent with previous studies demonstrating the beneficial effects of polyphenol-rich feed additives on egg quality and oxidative stability.

Egg weight increased significantly in the KLWP-supplemented groups, especially in K3 (6% KLWP), both during the 56-day feeding period and after 5 days of storage. This enhancement may be attributed to the phyto chemicals compounds in citrus waste, such as polyphenols, vitamin C, and essential oils, which are known to reduce oxidative stress and improve nutrient utilization (Eratak *et al*., 2023). Citrus-derived pectin may also slow intestinal transit, enhancing nutrient absorption and contributing to increased egg weight. Eggshell thickness was significantly higher in all KLWP-treated groups compared to the control. Polyphenols have been shown to improve eggshell quality by modulating calcium metabolism and enhancing mineral absorption (Liu *et al*., 2020). However, excessive polyphenol intake may impair digestive enzyme activity and mineral uptake, suggesting that optimal inclusion levels are critical.

Haugh unit, a key indicator of albumen quality and freshness, was markedly improved in KLWP groups, particularly K2 and K3. This aligns with findings from Yuan *et al*. (2016) and Wang *et al*. (2018), who reported that tea polyphenols enhance albumen height and HU values by stabilizing albumen proteins and reducing oxidative degradation. The antioxidant properties of KLWP likely contributed to the preservation of albumen integrity during storage, as evidenced by sustained HU values after 5 days. Interestingly, yolk index and eggshell weight remained unaffected across treatments, indicating that KLWP primarily influences albumen and shell matrix quality rather than yolk structure. This selective effect may be due to the differential distribution of polyphenols in reproductive tissues, with greater accumulation in the magnum (albumen-producing region) than in the ovary (yolk-producing region).

While KLWP inclusion showed promising results, caution is warranted regarding level of inclusion. High levels of polyphenols may interfere with digestive processes and trace mineral absorption, potentially compromising long-term egg quality and bird health (Nazok *et al*., 2010; Liu *et al*., 2020). Therefore, moderate inclusion rates (e.g., 2–4%) may offer optimal benefits without adverse effects. Overall, the findings suggest that KLWP is a viable functional feed additive for improving egg quality in laying quails. Its polyphenolic content enhances albumen stability, shell integrity, and egg weight, contributing to better shelf life and consumer appeal.

Polyphenol supplementation improves various aspects of egg quality. Tea polyphenols can enhance eggshell thickness, strength, and color, as well as albumen height and Haugh units, which are indicators of egg freshness and quality (Yuan *et al*., 2016; Wang *et al*., 2018). Yuan *et al*. (2016) demonstrated that incorporating tea polyphenols into the diet positively influenced egg freshness, as evidenced by higher albumen height and HU values. Polyphenols in tea can mitigate oxidative stress in laying hens, which is beneficial for maintaining egg production and quality under stress conditions, such as heat stress (Yuan *et al*., 2016)

Polyphenols enhance the Haugh unit through antioxidant activity, protein regulation, and preservation of albumen structural integrity. The strong positive correlation observed in this study suggests that the polyphenols present in KLWP likely exert a similar protective effect on albumen proteins. They may reduce oxidative degradation, resulting in more stable and elevated albumen, thereby improving the Haugh unit. Polyphenols interact with albumen proteins such as ovalbumin and lysozyme via hydrogen bonding and hydrophobic interactions. These reversible bonds help maintain protein structure and prevent unfolding or aggregation.

After consumption, polyphenols are absorbed in the intestine and enter the bloodstream (Zhou *et al*., 2012). Polyphenols can enhance antioxidant capacity, which might improve overall hen health and productivity. These active compounds are then transported to reproductive organs such as the ovary (responsible for yolk production) and the magnum (responsible for albumen/egg white formation). Polyphenols can accumulate in the yolk and influence albumen protein synthesis, antioxidant levels, and yolk coloration. Stabilization of albumen proteins leads to an increase in the Haugh unit of quail eggs. Additionally, higher antioxidant levels improve egg resistance to oxidation and physical degradation.The inclusion of a specific melon concentrate, which likely contains polyphenols, improved egg weight and yolk contribution to egg weight

After 5 d of storage, the K3 group (6% KLWP) exhibited a significantly higher egg weight than the control (0% KLWP) and K2 groups (4% KLWP), but no significant difference was observed when compared to the K1 group (2% KLWP). The Haugh Unit (HU) and shell thickness were highest in the K3 group (6% KLWP) compared to the control group (0% KLWP); however, no significant differences were observed between the K1 (2% KLWP) and K2 (4% KLWP) groups. The shell weight and yolk index of all groups with KLWP inclusion did not differ significantly ($p>0.05$) from those of the control group.

Abd El-Hack *et al*. (2023) stated that several specific polyphenols have been identified to enhance egg freshness, contributing to improved quality and shelf life. Dietary supplementation with tea polyphenols has been shown to positively affect egg freshness. This is evidenced by the increased albumen height and Haugh unit (HU) values, which are indicators of egg quality and freshness. The inclusion of tea polyphenols in the diet of laying hens can lead to better preservation of egg quality during storage. A layer chicken diet with high polyphenol levels obtained from extra virgin olive oil can enhance the fatty acid quality of egg yolk while lowering egg yolk cholesterol, which is important for consumers.

Although laying rate was the same between the groups (K0, K1, K2, and K3), egg weight increased significantly with the inclusion of KLWP. Adequate consumption of polyphenols, vitamin C, essential oils, and citrus waste can reduce stress levels in laying quails during production. Supplementation of quail diets with orange peel essential oil resulted in a significant increase in egg weight (Eratak *et al*., 2023). The highest egg mass was observed at 2% KLWP (K2), indicating an optimal balance between energy intake and nutrient retention. Increased pectin viscosity slows down the passage rate of ingesta in the digestive tract, reduces the rate of carbohydrate digestion, and maintains blood sugar stability. However, if the level of citrus waste is too high, bioactive compounds such as pectin and condensed tannins can inhibit the activity of digestive enzymes (Nazok *et al*., 2010).

The inclusion of high levels of polyphenols in poultry diets negatively affects digestive health. Excessive polyphenols may bind to biliary salts and suppress digestive enzymes, thereby impairing fat and protein digestion. This can ultimately affect egg quality by reducing nutrient availability Excessive polyphenol intake can lead to decreased eggshell strength and weight, potentially due to interactions with calcium metabolism (Liu *et al*., 2020). Additionally, polyphenols may alter the absorption of trace elements, such as copper, iron, and zinc, which are crucial for overall health and for egg quality. Polyphenols can also influence eggshell quality, which is an important aspect of overall egg quality.

## Conclusion

Overall, the findings suggest that Kasturi lime waste powder (KLWP) is a viable functional feed additive for improving egg quality in laying quails. Its polyphenolic content enhances albumen stability, shell integrity, and egg weight, contributing to better shelf life and consumer appeal. Moderate inclusion rates (e.g., 2–4%) appear to offer optimal benefits without adverse effects on protein digestion or trace mineral absorption.

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## Conflict of interest

The authors have no conflict of interest to declare.

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