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Effects of Fermented Fish Waste and Meat-bone Scraps on Laying Performance and Profitability in Japanese Quail

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ABSTRACT

Calcium is essential for quail growth and eggshell formation, but commercial sources are expensive. The present study aimed to evaluate fermented fish waste (FW) and meat and bone scraps (MBS) as alternative calcium sources for Japanese quail, examining their effects on growth, reproduction, egg production and quality, and economic returns. A total of 240 female quail (120 ± 10 g at 35 days post-hatch) were randomly allocated to eight dietary treatments (n = 30) in a randomized complete block design with three replications (10 quails each), comprising a formulated calcium-deficient diet (0.8% Ca of dry matter [DM]), a standard layer diet (3.0% Ca DM), and six calcium-supplemented diets using fermented substrates (50 or 100 mL/kg, sprayed onto basal feed). The feeding trial lasted 85 days and evaluated growth performance, reproductive and productive traits, egg quality, and profitability. Mineral analysis of the fermented substrates showed that MBS contained significantly higher calcium than FW (14.86 versus 11.12 mg/kg), while phosphorus and iron levels were comparable. Growth performance differed significantly among treatments at 70 (mid-lay) and 120 (peak production) days post-hatch, with 50 mL MBS/kg producing the highest body weights (180.28 g at 70 days; 229.48 g at 120 days). Reproductive traits (age at first egg, age at sexual maturity, and body weight at maturity) were unaffected by dietary treatments. Production performance and egg quality improved significantly with calcium supplementation compared to the calcium-deficient control. At 120 days, 50 mL MBS yielded the highest hen-day production (96.82%), egg weight (10.83 g), and egg mass (41.94 g) compared to all other treatments, including the standard diet. Shell thickness and albumen height with 50 mL MBS significantly exceeded those of the calcium-deficient diet and matched or surpassed those of the standard diet at both measurement times. Economic analysis showed the highest return on investment for 50 mL MBS (68.69%), comparable to the standard diet (66.06%). These results demonstrate that 50 mL/kg fermented MBS provides a practical, sustainable, and cost-effective calcium source for laying quail, achieving optimal performance across growth, production, egg quality, and economic parameters under the conditions tested.

Keywords: Bone scrap, Calcium supplementation, Circular economy, Egg production, Egg quality, Fermented fish waste, Japanese quail

INTRODUCTION

Calcium is essential in poultry diets due to its critical roles in skeletal development (Li et al., 2017; Torres and Korver, 2018; David et al., 2023), eggshell formation (Sah and Mishra, 2018), neuromuscular regulation (Gehlert et al., 2015; Zhu and Xiao, 2025), and enzyme activation (Proszkowiec-Weglarz and Angel, 2013; Bedford and Rousseau, 2017; Vertiprakhov et al., 2021). As the most abundant mineral in avian physiology, calcium is vital for bone remodeling, synaptic transmission, and intracellular signaling (Wilkinson et al., 2011). In laying birds, inadequate calcium impairs egg production (Chen et al., 2020; Zhao et al., 2020), produces fragile shells (Jiang et al., 2013; Chen et al., 2015), reduces egg mass, and compromises skeletal integrity, leading to bone mobilization and increased risk of layer fatigue (Mavromichalis, 2016). Commercial layer production typically relies on limestone, oyster shell, or dicalcium phosphate supplements (Sah and Mishra, 2018; Diana et al., 2021). However, rising costs, supply instability, and environmental impacts of mineral extraction raise long-term sustainability concerns (Mavromichalis, 2016; Patil et al., 2024). These challenges have intensified the search for alternative, cost-effective, and renewable calcium sources that align with circular bioeconomy principles.

Among emerging alternatives, fermented organic by-products offer a dual advantage by providing bioavailable nutrients (Faria et al., 2023) while addressing waste management challenges (He and Cui, 2025). The conversion of waste materials into feed ingredients through microbial fermentation represents an opportunity to close nutrient loops in agricultural systems (Chilakamarry et al., 2022), reduce dependence on mined minerals (Yasmeen and Ahmad, 2025), and mitigate environmental impacts associated with both waste disposal and mineral extraction (Ahmad et al., 2023; He and Cui, 2025).

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Fermentation of organic waste has gained attention as a cost-effective strategy to enhance nutrient bioavailability, reduce anti-nutritional factors, and generate value-added feed materials enriched with microbial metabolites and bioactive compounds (such as organic acids, amino acids, peptides, and probiotic microorganisms; Ahmad et al., 2023; Yafetto et al., 2023; Saeed et al., 2025). Fish waste (FW) and meat-bone residues are mineral-rich by-products of food processing (Yessimbekov et al., 2021; Zhang et al., 2023; Khairul et al., 2024). In the Philippines, inadequate use of these materials contributes to landfill waste accumulation and environmental pollution through methane emissions and leachate contamination (De Ungria et al., 2021; 2023). Wet markets in Metro Manila, the Philippines, generate an average of 70.3 kg of FW per market daily and at least 1.8 tons annually. Fish by-products comprise 20-80% of processed fish but are often discarded rather than recovered as feed resources (Islam et al., 2021; Patil et al., 2024). Similarly, meat and bone scraps (MBS) from Philippine local markets remain underutilized despite their potential as feedstock for nutrient recovery (ADB and IFAD, 2019; Graham, 2022). Fermenting these materials into liquid nutrient formulations offers a means to recover bioavailable minerals while supporting waste reduction and circular economy principles (Tang et al., 2018; Pawar et al., 2020; Patil et al., 2024). However, scientific evaluation of fermented FW and MBS as calcium supplements in quail diets remains limited, despite quail's high calcium turnover and early maturity (Bar, 2009).

Although several alternative calcium sources, including eggshell powder (Zhao et al., 2020), snail and mollusk shells (Mavromichalis, 2016; Anizoba et al., 2021), and dicalcium phosphate (Cheng et al., 2019; Diana et al., 2021) have been investigated, evidence on fermented animal waste remains scarce (Samaddar, 2018; Yafetto et al., 2023). To date, no study has directly compared fermented FWs and meat-bone scraps, characterized their mineral profiles, or evaluated their impacts on growth, reproduction, egg production, and egg quality in quail. Economic profitability is also rarely incorporated into calcium supplementation studies. Addressing these knowledge gaps is essential to identify sustainable mineral sources that reduce dependence on mined supplements, mitigate waste accumulation, and promote nutrient recovery from processing by-products in poultry production. This study evaluated fermented FW and MBS as alternative calcium sources for laying quail and assessed their effects on nutritional value, productivity, and economic viability.

MATERIALS AND METHODS

Ethical approval

Before the study was initiated, ethical approval was obtained from the study ethics committee of Agusan del Sur State College of Agriculture and Technology (ASSCAT), Bunawan, Agusan del Sur, Mindanao, Philippines. All procedures complied with the Philippines Republic Act No. 8485, otherwise known as the "Animal Welfare Act of 1998".

Experimental location

The study was conducted at West Bunawan National High School, Bunawan, Agusan del Sur, Mindanao, Philippines, from July to November 2025. The site experiences a Type II climate (no dry season with very pronounced maximum rainfall from November to January), with a mean annual precipitation of 3,500 mm and a mean annual temperature of 27.5°C (Philippine Atmospheric, Geophysical and Astronomical Services Administration, 2024).

Preparation and fermentation of substrates

Fresh fish tamban (local name for *Sardinella albella*, a small pelagic fish species) and mixed meat-bone scraps (chicken, beef, pork) sourced from Butuan City Public Market, Butuan City, Philippines. All materials were transported in insulated containers (2-4°C) and filleted or chopped within 2 h to maintain freshness. Fish were filleted to collect waste materials (heads, bones, viscera, scales), and meat-bone scraps were manually chopped into 2-4 cm pieces. All materials were then washed with potable water (25°C) and drained for 30 min in stainless-steel mesh containers (2 mm mesh) at ambient temperature (28 ± 2°C). Processed substrates were mixed separately with commercial molasses (Brix 79-81°) and distilled water at a 1:1:1 ratio by weight in sanitized 20 L high-density polyethylene (HDPE) containers (Salas, 2024). Fermentation lasted 30 days at 27 ± 2°C and 65 ± 5% relative humidity (RH), with daily pH monitoring (4.0-4.5). After fermentation, the liquid fraction was filtered through sterile cheesecloth and stored at 4°C in amber glass bottles until use.

Experimental animals, housing, and design

Three hundred day-old Japanese quail (*Coturnix coturnix japonica*) were brooded communally for 30 days with decreasing temperature (35°C in week 1, then reduced 3°C weekly). Quails were vaccinated against Newcastle disease at

day 30 using the LaSota strain live vaccine (VAKSINDO, Bogor, Indonesia) administered via the ocular route. At 35 days post-hatch, 240 female quails (120 ± 10 g) were randomly allocated to eight treatments with three replication blocks (n = 30 per treatment; 10 quails per replicate). Treatment groups included T_1 = calcium-deficient diet (CD, 0.8% Ca); T_2 = standard layer diet (SD, 3.0% Ca); T_3 = CD + 50 mL fermented FW/kg; T_4 = CD + 50 mL fermented meat-bone scraps (MBS)/kg; T_5 = CD + 25 mL FW + 25 mL MBS/kg; T_6 = CD + 100 mL FW/kg; T_7 = CD + 100 mL MBS/kg; T_8 = CD + 50 mL FW + 50 mL MBS/kg. Quails were housed in galvanized wire cages, each providing 135 cm² per quail, with linear feeders and nipple drinkers. Lighting followed a 16L:8D photoperiod at 20–25 lux. Environmental temperature and relative humidity averaged 27.8 ± 2.3°C and 68.5 ± 6.2%, respectively, recorded by digital data loggers.

Dietary treatments and feeding management

The calcium-deficient basal diet contained 0.8 ± 0.05% Ca, while the standard layer diet contained 3.0 ± 0.05% Ca, formulated according to nutritional requirements for laying quail (NRC, 1994; Table 1). Fermented liquid supplements were measured (50 or 100 mL/kg) using graduated cylinders (±0.1 mL precision) and sprayed uniformly onto the basal feed before each feeding (07:00 and 16:00 h) for 85 days (from 35 to 120 days post-hatch), allowing 10 min for absorption. Daily feed intake was monitored by weighing offered and residual feed using a calibrated digital balance (±0.01 g precision), with spillage collected, dried (65°C, 24 h), and subtracted from consumption. Water was provided *ad libitum*.

Table 1. Ingredient composition and proximate analysis of standard layer and calcium deficient diet of Japanese quail

Ingredients (%, as-fed basis)	Standard calcium diet	Calcium deficient
Corn (kg)	45	50
Wheat (kg)	8	8
Soybean meal (kg)	14	15
Vegetable oil (kg)	3	3
Corn gluten (kg)	5	5
Corn germ meal (kg)	2	2
Corn bran (kg)	2	2
Pollard (kg)	2	2
Copra meal (kg)	3	3
Rice bran (kg)	2	2
Dried distillers (kg)	2	2
Molasses (kg)	2	2
Limestone (kg)	6	1
Mono-dicalcium phosphate (kg)	3	2
Salt (kg)	0.5	0.5
Feed premix (kg)	0.5	0.5
TOTAL (kg)	100	100
Chemical analysis		
Crude protein (%)	20.30	20.00
Crude fat (%)	5.00	5.00
Crude fiber (%)	4.00	4.00
Calcium (%)	3.00	0.80
Phosphorus (%)	0.70	0.70
Moisture (%)	12.00	12.00

Feed premix provided per kg of diet: Vitamin A, 10,000 IU; Vitamin D_3, 2,000 IU; Vitamin E, 10 mg; Vitamin K_3, 2 mg; Vitamin B_1, 1 mg; Vitamin B_2, 4 mg; Vitamin B_{12}, 0.01 mg; Niacin, 20 mg; Biotin, 0.05 mg; Folic acid, 0.5 mg; Pantothenic acid, 10 mg; Choline chloride, 300 mg; Manganese, 60 mg; Copper, 5 mg; Magnesium, 300 mg; Iron, 40 mg; Zinc, 50 mg; Iodine, 0.3 mg; Selenium, 0.15 mg

Data collection and measurements

Growth and reproduction

Individual body weights were recorded at 35, 42, 70, and 120 days post-hatch using a digital balance (±0.01 g). Age at first egg (AFE) was recorded when the first egg was laid per replicate, and quails were weighed on that day to determine body weight at sexual maturity. Age at sexual maturity (ASM) was calculated by the following formula (Leeson and Summers, 2005).

ASM = Age at first egg (AFE) + [(0.5 / rate of lay in following week) × 7]

Egg production

Eggs were collected twice daily (08:00 and 16:00 h). Hen-day egg production percentage (HDPP) was calculated at 70- and 120-days post-hatch, where HDPP (%) equals total eggs divided by total hens multiplied by the total eggs divided by the total hens multiplied by the number of days, then multiplied by 100 (Gous and Morris, 2005). Egg weight was measured within 2 h of collection using a digital balance (±0.01 g). Egg mass (g) was calculated as HDPP multiplied by average egg weight. Feed-egg-ratio was determined as total feed consumed divided by total egg mass produced (Chimezie et al., 2017).

Egg quality

Egg quality assessments were conducted along with egg production (70 and 120 days post-hatch. Shell thickness was measured at the sharp end, blunt end, and equator using a digital micrometer (±0.001 mm resolution) after membrane removal. The egg shape index was determined according to the following formula of Duman et al. (2017).

Egg shape index (%) = (egg width / egg length) × 100

Albumen height was measured using a digital height gauge (± 0.01 mm) at the midpoint between the yolk edge and the outer albumen margin. Yolk index was calculated as the ratio of yolk height to yolk diameter (Kul and Şeker, 2004).

Economic analysis

Economic profitability was evaluated by calculating gross revenue (total eggs × market price in Philippine Peso, PHP), net profit (gross revenue minus total costs), and return on investment (ROI, %) as net profit divided by total costs and multiplied by 100 (Nwude, 2012). Total costs included chicks, feed, fermented supplements, vaccination, medication, utilities, and labor.

Chemical analyses

Mineral composition of fermented FW and meat-bone scraps was determined at the Regional Analytical Laboratory, Department of Agriculture – Regional Field Office XIII, Butuan City, Philippines. Samples (5 g) underwent wet acid digestion (HNO_3:$HClO_4$, 3:1 v/v) at 150°C following standard methods (AOAC, 2019). Calcium was determined by the dry ash method (AOAC method 968.08). Phosphorus was determined spectrophotometrically at 880 nm (AOAC method 995.11). Magnesium and iron were analyzed by atomic absorption spectrophotometry (Shimadzu AA-7000, Japan) following the AOAC method 999.11. Results were expressed on a dry weight basis (mg/kg for calcium, magnesium, and iron; mg/g for phosphorus) after moisture determination (105°C, 24 h).

Statistical analysis

The mineral composition of fermented materials was compared using an independent samples t-test. Experimental data were analyzed using one-way ANOVA with treatment as a fixed effect and replicate pen as the experimental unit (SPSS version 25.0, IBM Corp., Armonk, NY, USA). Data normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. When significant effects were detected ($p < 0.05$), means were separated using Tukey's HSD test at $p < 0.05$.

RESULTS AND DISCUSSION

Mineral characteristics

Mineral composition data (Table 2) showed significantly higher Ca concentrations in MBS compared to FW ($p < 0.001$), while P, Fe, and Mg did not differ significantly between substrates ($p > 0.05$). This elevated Ca concentration reflects the greater hydroxyapatite density of mammalian and avian long-bone tissues (Field, 2000; Torres and Korver, 2018) compared to the lower mineral density of small pelagic fish bones (Egerton et al., 2020; Cohen et al., 2021). Fermented MBS consisted predominantly of cortical vertebrae and long bones with high mineral density (Yessimbekov et al., 2021), whereas FW was composed of mixed teleost bones and soft tissues (Fiedler et al., 2019), explaining the 33.6% difference in Ca concentration.

Table 2. Mineral content of fermented fish and meat waste and bone scraps (Philippines, 2025)

Constituents	Calcium (mg/kg)	Phosphorus (mg/g)	Magnesium (mg/kg)	Iron (mg/kg)
MBS	14.86 ± 0.190^{b}	2.09 ± 0.250	< DL	2.07 ± 0.242
FW	11.12 ± 0.220^{a}	1.59 ± 0.010	< DL	1.88 ± 0.675
P-value	< 0.001***	0.068^{ns}	--	0.074^{ns}

The different superscript letters in a column mean statistically significant difference at p value less than 0.05; *** Very highly significant at p value less than 0.001; ^{ns} Not significant at 0.05 levels; < DL: Below detection limit; FW: Fermented fish waste; MBS: Fermented meat and bone scraps

Fermentation further enhanced Ca release via acidification by lactic acid bacteria (pH ~4–4.5), which accelerated hydroxyapatite dissolution and the formation of soluble Ca^{2+}–organic acid complexes (Tang et al., 2018; Han et al., 2018; Meng et al., 2023). Although mammalian bone dissolved more slowly, its higher mineral density enabled greater Ca solubilization under acidogenic conditions, as demonstrated by biotic–abiotic dissolution profiles (Salek et al., 2013). Similar P levels across substrates reflected limited phosphate solubilization and retention within protein–mineral matrices (Bhaskar et al., 2007; Chalamaiah et al., 2012), while comparable Fe levels were consistent with Fe being primarily associated with heme proteins in soft tissues rather than in bone (Hurrell and Egli, 2010). Consequently, MBS provided higher bioavailable Ca while maintaining P and Fe levels comparable to FW.

Growth performance

Body weight did not differ among treatments at 35 or 42 days (Table 3), indicating that the short Ca-deficient feeding window (35 to 42 days) permitted somatic growth through osteoclastic mobilization despite early Ca depletion ($p > 0.05$) (Costa et al., 2009; Pourmollaei et al., 2025). By 70 and 120 days post-hatch, significant treatment effects emerged as the onset of sexual maturity increased the Ca demand for medullary bone and shell formation ($p < 0.001$) (Kim et al., 2012). Prolonged Ca deficiency intensified bone resorption and disrupted endocrine regulation (Li et al., 2020), depressing growth as skeletal quality deteriorated. The 50 mL MBS/kg diet produced the highest body weights (180.28 g at 70 d; 229.48 g at 120 d), reflecting its higher hydroxyapatite-derived Ca content (Field, 2000; Torres and Korver, 2018) and bone composition enriched with cortical vertebrae and long bones (Yessimbekov et al., 2021). This contrasted with the lower and more variable mineral content of teleost-sourced FW (Fiedler et al., 2019; Egerton et al., 2020; Cohen et al., 2021), which limited its effectiveness as a calcium source.

Fermentation enhanced MBS efficacy by driving lactic acid bacterial acidification that dissolved hydroxyapatite, thereby increasing free Ca^{2+} and Ca–organic acid complexes, while proteolysis released minerals and peptides (Han et al., 2018; Meng et al., 2023). These mechanisms explained the consistent superiority of 50 mL MBS. The dose-response pattern showing optimal performance at 50 mL but declining at 100 mL (inverted-U curve) indicated hormesis, whereby excessive Ca at 100 mL disrupted the Ca:P balance and impaired digestibility (Calabrese, 2003; An et al., 2016). Fermented fish waste yielded modest improvements due to its low ash density and higher cartilage and lipid content of pelagic waste, which limit mineral recovery and fermentation efficiency (Toppe et al., 2007; Fiedler et al., 2019). Combined fermented FW plus MBS treatments showed intermediate responses, suggesting non-complementary co-fermentation likely driven by substrate dilution and reduced lactic acid bacterial acidification (Han et al., 2018; Tang et al., 2018). Quail fed 50 mL MBS maintained stable weights from 70 to 120 days, consistent with enhanced nutrient release, increased peptide availability, and improved gut morphology documented for fermented substrates (Shabani et al., 2018; Guimarães et al., 2019; Shabani et al., 2021). In contrast, Ca-deficient quail exhibited weight stagnation, reflecting constrained skeletal mobilization and reduced metabolic efficiency (Table 3).

Table 3. Body weight (g) of Japanese quail at different ages fed low calcium diets supplemented with fermented fish waste and meat and bone scraps (Philippines, 2025)

Treatment	35 days	42 days	70 days	120 days
CD (no application)	109.87 ± 4.34	136.53 ± 5.74	143.13 ± 2.20^{a}	146.53 ± 3.18^{ab}
SD (no application)	110.93 ± 1.50	136.40 ± 2.70	148.66 ± 6.70^{ab}	147.00 ± 4.35^{ab}
CD + 50 ml FW	109.20 ± 1.60	136.33 ± 1.47	152.40 ± 7.00^{ab}	157.67 ± 7.37^{ab}
CD + 50 ml MBS	111.07 ± 3.06	139.80 ± 5.52	184.93 ± 11.95^{c}	184.93 ± 12.37^{c}
CD + 25 ml FW + 25 ml MBS	112.53 ± 1.00	135.46 ± 9.79	147.4 ± 4.88^{ab}	146.80 ± 2.94^{ab}
CD + 100 ml FW	112.20 ± 3.63	133.47 ± 12.30	160.20 ± 13.74^{ab}	153.53 ± 3.31^{ab}
CD + 100 ml MBS	111.33 ± 2.80	136.53 ± 10.81	168.67 ± 9.01^{cb}	166.00 ± 11.13^{bc}
CD + 50 ml FW + 50 ml MBS	117.47 ± 6.18	130.07 ± 7.80	145.07 ± 2.53^{a}	142.43 ± 2.35^{p}
P - value	0.198^{ns}	0.894^{ns}	< 0.001***	< 0.001***

The different superscript letters in a column mean statistically significant difference at p value less than 0.05; *** Very highly significant at p value less than 0.001; ^{ns} Not significant at 0.05 level; CD: Calcium deficient; SD: Standard calcium diet; FW: Fermented fish waste; MBS: Fermented meat and bone scraps; The data are expressed as mean ± Standard deviation

Reproduction performance

Age at first egg (AFE), age at sexual maturity (ASM), and maturity body weight did not differ among treatments ($p > 0.05$). Age at first egg (36-38 d), ASM (37-39 d), and body weights (105-118 g) remained within expected biological ranges for Japanese quail (Sezer et al., 2006; Retes et al., 2022), indicating that Ca source or level during the short pre-lay window did not modify reproductive onset (Table 4). Sexual maturation in quail is driven by photoperiodic activation

of the hypothalamic-pituitary-gonadal (HPG) axis, whereby gonadotropin-releasing hormone (GnRH) triggers follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion, which in turn drives rapid follicular recruitment (Dawson et al., 2001; Zhou et al., 2023), provided that minimum body weight and energy–protein thresholds are met (Retes et al., 2022; Vogado et al., 2024). Because pre-lay ovarian and oviductal development requires minimal Ca relative to the demands of eggshell calcification after the onset of lay, short-term differences in Ca supply had limited capacity to shift AFE or ASM.

The narrow AFE–ASM interval and low variability across treatments showed that reproductive initiation followed genetically programmed photoperiodic timing rather than nutrient modulation (Sezer et al., 2006; Vogado et al., 2024). Key threshold traits, particularly lipid reserves and lean mass, governed activation of the reproductive cascade (Retes et al., 2022; Vogado et al., 2024), and these traits were determined by earlier growth phase energy–protein nutrition rather than by micronutrient variation before lay. Since the experimental diets were introduced at 35 days post-hatch, after the critical developmental window for reproductive organ formation (Retes et al., 2022), the Ca treatments had no opportunity to influence the HPG axis trajectory. Consequently, flock maturity progressed uniformly across all diets, confirming that photoperiod and growth-phase nutrients determined the timing of reproductive onset in Japanese quail.

Table 4. Age at first egg (days), age at sexual maturity (days), and maturity body weight (g) of quail supplemented with fermented fish waste and meat and bone scraps (Philippines, 2025)

Treatment	Age at first egg (d)	Age at sexual maturity (d)	Maturity body weight (g)
CD (no application)	36.67 ± 1.54	37.77 ± 1.18	109.81 ± 4.36
SD (no application)	36.67 ± 1.55	37.77 ± 1.18	110.97 ± 1.60
CD + 50 ml FW	37.33 ± 0.57	38.45 ± 0.59	118.26 ± 17.11
CD + 50 ml MBS	37.00 ± 1.73	38.11 ± 1.78	105.45 ± 1.99
CD + 25 ml FW + 25 ml MBS	37.67 ± 2.08	38.79 ± 2.14	116.30 ± 7.54
CD + 100 ml FW	37.00 ± 1.00	38.11 ± 1.03	112.21 ± 3.64
CD + 100 ml MBS	37.00 ± 1.00	38.11 ± 1.03	111.32 ± 2.71
CD + 50 ml FW + 50 ml MBS	36.00 ± 0.00	37.08 ± 0.00	111.93 ± 3.50
P - value	0.836^{ns}	0.836^{ns}	0.532^{ns}

^{ns} Not significant at $p < 0.05$; CD: Calcium deficient; SD: Standard calcium diet; FW: Fermented fish waste; MBS: Fermented meat and bone scraps; The data are expressed as mean ± Standard deviation

Production performance

Dietary Ca source significantly affected all production traits at 70 and 120 days ($p < 0.001$, Table 5). At 70 days, quails fed the standard diet outperformed those on the Ca-deficient control, confirming the sensitivity of egg production and egg mass to Ca adequacy during the early laying stage (Chen et al., 2020; Zhao et al., 2020). By 120 days, 50 mL MBS yielded the highest hen-day production, egg weight, and egg mass, surpassing both the standard diet and all other Ca sources. Production in the Ca-deficient control remained significantly lower due to medullary bone depletion once labile Ca reserves were exhausted, leaving no mobilizable calcium for eggshell formation (Kim et al., 2012; Zhao et al., 2020). Fermented fish waste produced intermediate improvements, which aligned with its lower Ca density. In contrast, FW combined with MBS showed no synergistic effects, indicating that FW's nutritional constraints limited the gains from MBS. This lack of additivity suggests that the lower mineral density and fermentation efficiency of FW prevented the MBS benefits when the two substrates were combined.

The strong response to 50 mL MBS reflects its high mineral density, particularly calcium and phosphorus, as confirmed by mineral analysis (Table 2). Additionally, fermentation likely increased Ca solubility, peptide formation, and organic-acid chelation, thereby enhancing absorption and gut integrity (Shabani et al., 2018; 2021). These mechanisms improved egg weight and mass, both of which are linked to nutrient assimilation and follicular development (Proszkowiec-Weglarz and Angel, 2013; Chen et al., 2015). In this study, Ca deficiency reduced egg weight by ~15%, consistent with impaired follicular development (Chen et al., 2020; Zhao et al., 2020), while poor performance in the Ca-deficient control reflected medullary bone depletion despite behavioral Ca-intake regulation (Kim et al., 2012; Zhao et al., 2020). The dose-response pattern, whereby 50 mL outperformed 100 mL MBS, demonstrated the inverted-U relationship characteristic of fermented feed supplementation. This pattern aligns with previously documented optimal inclusion levels for fermented feeds, where excess fermentation biomass or acid load depresses performance (Boitai et al., 2018; Panda, 2017). In the current study, the improvement achieved with 50 mL of MBS exceeded responses to conventional Ca supplements, while offering lower production costs. These benefits highlight 50 mL MBS/kg as a biologically and economically superior Ca source.

Table 5. Production performance of quail at 70 and 120 days supplemented with fermented fish waste and meat and bone scraps (Philippines, 2025)

Treatment	HDPP 70 (pc)	HDPP 120 (pc)	Egg weight 70 (g)	Egg weight 120 (g)	Feed-egg-ratio 70	Feed-egg-ratio 120	Egg mass 70	Egg mass 120
CD (no application)	61.60 ± 0.06^{a}	75.22 ± 0.01^{a}	7.97 ± 0.22^{a}	9.42 ± 0.08^{a}	0.41 ± 0.04^{ab}	0.33 ± 0.01^{b}	19.66 ± 2.51^{a}	28.33 ± 0.59^{a}
SD (no application)	84.45 ± 0.05^{b}	95.33 ± 0.03^{b}	9.15 ± 0.23^{bc}	10.02 ± 0.15^{ab}	0.30 ± 0.01^{a}	0.26 ± 0.01^{a}	30.95 ± 2.32^{b}	38.23 ± 1.67^{bc}
CD + 50 ml FW	59.84 ± 0.04^{a}	86.96 ± 0.11^{ab}	8.44 ± 0.20^{ab}	10.17 ± 0.19^{b}	0.42 ± 0.03^{b}	0.29 ± 0.04^{ab}	20.19 ± 0.90^{a}	35.29 ± 3.98^{abc}
CD + 50 ml MBS	70.49 ± 0.08^{ab}	96.82 ± 0.01^{b}	9.67 ± 0.79^{c}	10.83 ± 0.17^{c}	0.36 ± 0.4^{ab}	0.25 ± 0.00^{ab}	27.44 ± 5.64^{ab}	41.94 ± 0.66^{c}
CD + 25 ml FW + 25 ml MBS	57.63 ± 0.09^{a}	80.72 ± 0.07^{ab}	8.59 ± 0.25^{abc}	10.14 ± 0.44^{b}	0.44 ± 0.07^{b}	0.30 ± 0.03^{ab}	19.88 ± 3.79^{a}	34.59 ± 4.20^{abc}
CD + 100 ml FW	58.67 ± 0.00^{a}	84.96 ± 0.07^{ab}	8.42 ± 0.49^{ab}	9.67 ± 0.08^{ab}	0.43 ± 0.00^{b}	0.30 ± 0.03^{ab}	19.77 ± 1.23^{a}	32.87 ± 3.04^{ab}
CD + 100 ml MBS	69.21 ± 0.10^{ab}	83.03 ± 0.03^{ab}	8.67 ± 0.39^{abc}	9.89 ± 0.19^{ab}	0.37 ± 0.06^{ab}	0.30 ± 0.01^{ab}	24.13 ± 4.79^{ab}	32.85 ± 1.19^{ab}
CD + 50 ml FW + 50 ml MBS	54.19 ± 0.03^{a}	80.72 ± 0.11^{abc}	8.59 ± 0.33^{abc}	10.04 ± 0.19^{ab}	0.46 ± 0.03^{b}	0.31 ± 0.04^{ab}	18.58 ± 0.57^{a}	32.35 ± 3.95^{ab}
P - Value	0.001**	0.029*	0.005**	< 0.001***	0.004**	0.050*	0.002**	0.001**

^{abc} The different superscript letters in a column mean statistically significant difference at p value less than 0.05; *** Very highly significant at p value less than 0.001; ** Highly significant at p value less than 0.01; * Significant at p < 0.05; CD: Calcium deficient; SD: Standard calcium diet; FW: Fermented fish waste; MBS: Fermented meat and bone scraps; HDPP 70: Hen day egg production percentage at 70 days post hatch: HDPP 120: Hen day egg production percentage at 120 days post hatch; The data are expressed as mean ± Standard deviation

Table 6. Egg quality traits of quail at 70 and 120 days supplemented with fermented fish waste and meat and bone scraps (Philippines, 2025)

Treatment	Shell thickness 70	Shell thickness 120	ESI 70	ESI 120	Albumen height 70	Albumen height 120	Yolk index 70	Yolk index 120
CD (no application)	0.079 ± 0.07^{a}	0.318 ± 0.014^{a}	81.11 ± 0.96	82.38 ± 0.80	3.51 ± 0.21^{a}	3.27 ± 0.15^{a}	0.384 ± 0.016	0.300 ± 0.023^{a}
SD (no application)	0.112 ± 0.006^{c}	0.422 ± 0.007^{c}	83.67 ± 6.70	80.08 ± 3.66	4.45 ± 0.15^{b}	4.58 ± 0.51^{b}	0.405 ± 0.0110	0.360 ± 0.020^{b}
CD + 50 ml FW	0.106 ± 0.001^{bc}	0.373 ± 0.014^{b}	83.02 ± 3.38	79.78 ± 1.86	4.65 ± 0.32^{b}	5.14 ± 0.15^{bc}	0.413 ± 0.028	0.398 ± 0.016^{bc}
CD + 50 ml MBS	0.140 ± 0.005^{d}	0.419 ± 0.017^{c}	84.55 ± 0.33	78.86 ± 0.50	5.18 ± 0.17^{b}	5.43 ± 0.47^{bc}	0.425 ± 0.020	0.377 ± 0.019^{bc}
CD + 25 ml FW + 25 ml MBS	0.098 ± 0.003^{bc}	0.364 ± 0.017^{b}	80.03 ± 1.88	79.39 ± 2.70	5.11 ± 0.43^{b}	4.72 ± 0.31^{bc}	0.400 ± 0.029	0.373 ± 0.009^{bc}
CD + 100 ml FW	0.098 ± 0.005^{bc}	0.366 ± 0.015^{b}	81.64 ± 0.56	83.94 ± 4.06	4.36 ± 0.11^{b}	5.47 ± 0.23^{bc}	0.392 ± 0.036	0.411 ± 0.011^{c}
CD + 100 ml MBS	0.095 ± 0.008^{ab}	0.371 ± 0.016^{b}	82.74 ± 1.57	82.16 ± 0.60	5.02 ± 0.51^{b}	5.62 ± 0.34^{c}	0.400 ± 0.013	0.385 ± 0.020^{bc}
CD + 50 ml FW + 50 ml MBS	0.096 ± 0.007^{bc}	0.352 ± 0.019^{ab}	81.41 ± 4.19	80.56 ± 1.79	4.85 ± 0.12^{b}	5.46 ± 0.34^{bc}	0.418 ± 0.011	0.388 ± 0.019^{bc}
P - Value	< 0.001***	< 0.001***	0.707^{ns}	0.197^{ns}	< 0.001***	< 0.001***	0.412^{ns}	< 0.001***

^{abc} The different superscript letters in a column mean statistically significant difference at p value less than 0.05; *** Very highly significant at p value less than 0.001; ^{ns} Not significant; CD: Calcium deficient; SD: Standard calcium diet; FW: Fermented fish waste; MBS: Fermented meat and bone scraps; ESI: Egg shape index. The data are expressed as mean ± Standard deviation

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Egg quality

Significant effects on shell thickness were detected at both ages, with 50 mL MBS producing the thickest shells at 70 days and both 50 mL MBS and the standard diet remaining superior to Ca-deficient quail at 120 days ($p < 0.001$; Table 6). These patterns reflected the dependence of calcite deposition on dietary Ca, where deficiency reduced shell thickness (Jiang et al., 2013; Chen et al., 2015), while adequate Ca improved shell structure (Jiang et al., 2013; Chen et al., 2020), consistent with the high Ca availability of oyster shell relative to limestone (Jiang et al., 2013). Albumen height was also higher, with 50 mL MBS and FW+MBS yielding the highest values at 70 days and 100 mL MBS and 100 mL FW producing the greatest gains at 120 days, indicating improved protein deposition and metabolic support when Ca requirements were met ($p < 0.001$; Proszkowiec-Weglarz and Angel, 2013). Yolk index differed only at 120 days, peaking at 100 mL FW (0.411), consistent with slower vitelline-membrane remodeling and the enhanced release of peptides and amino acids during fish-waste fermentation (Aspmo et al., 2005; Olsen and Toppe, 2017). Egg shape index remained unaffected, indicating strong genetic control. Across all production and egg quality traits, 50 mL MBS generated the most consistent improvements. At the same time, Ca-deficient quail showed severe reductions of up to 59% thinner shells and 72% lower albumen height, reflecting impaired Ca homeostasis and medullary bone depletion (Jiang et al., 2013; Chen et al., 2020).

The superior performance of 50 mL MBS reflected fermentation effects that increased Ca solubility and organic-acid release (Shabani et al., 2018; Khairul et al., 2024), coupled with improvements in intestinal villus architecture and nutrient absorption mediated by lactic acid bacteria (Shabani et al., 2021). Meat and bone substrates supplied high Ca and P densities (Yessimbekov et al., 2021), providing precursors for shell calcification and medullary bone turnover (Kim et al., 2012; Zhao et al., 2020). The high mineral density of MBS explained the superior shell outcomes relative to FW. Enhanced albumen height aligned with increased peptide release and proteolysis during fermentation (Aspmo et al., 2005) and coincided with reports that low-level fermented fish silage maintains egg quality (Collazos and Guio, 2007; Guimarães et al., 2019). The 100 mL FW rise in yolk index reflected peptide stabilization of vitelline membranes rather than Ca effects. The decline in performance at 100 mL MBS and FW corresponded to the inverted-U response pattern observed in fermented feeds, in which excessive acid load or microbial solids reduced nutrient absorption efficiency (Collazos and Guio, 2007; Panda, 2017; Guimarães et al., 2019). These results highlighted fermented MBS as a bioavailable Ca source that could match or exceed conventional supplements and emphasize shell and albumen traits as sensitive biomarkers of Ca status and fermentation-mediated improvements in nutrient bioavailability.

Economic profitability

Economic indicators differed significantly among treatments, with the standard diet and 50 mL MBS producing the highest gross sales, net profit, and ROI (66.06% and 68.69%, respectively), while Ca-deficient quail returned only 39% ($p < 0.01$; Table 7). The strong economic response to 50 mL MBS reflected the conversion of biological improvements into revenue, as adequate Ca enhances shell mass and stability (Jiang et al., 2013; Chen et al., 2020). Commercial layer diets typically contain 36-40 g Ca/kg to meet the demands of sustained eggshell formation (Bar et al., 2002), and improvements in egg production and shell quality occur when dietary Ca levels approach these concentrations through bioavailable sources such as fermented MBS. Consequently, gross revenue with 50 mL MBS exceeded the Ca-deficient control by 27%, and net profit by 69%. Although not statistically different, 50 mL MBS showed numerically higher ROI than the standard diet due to lower substrate cost, enhanced digestibility and villus development (Shabani et al., 2018; 2021), and reduced breakage losses—consistent with reports that fermented fish silage ≤ 6% maintains egg quality and performance in quail (Collazos and Guio, 2007).

Table 7. Economic indicators of quail production supplemented with fermented fish waste and meat and bone scraps (Philippines, 2025)

Treatment	Gross sales (PHP)	Net sales (PHP)	ROI (%)
CD (no application)	1119.17 ± 103.00^{a}	308.17 ± 103.00^{a}	39.00 ± 12.70^{ab}
SD (no application)	1488.33 ± 101.31^{c}	592.08 ± 101.31^{b}	66.06 ± 11.30^{bc}
CD + 50 ml FW	1190.83 ± 79.43^{ab}	348.08 ± 79.43^{ab}	41.30 ± 9.42^{abc}
CD + 50 ml MBS	1421.66 ± 83.75^{bc}	578.92 ± 83.75^{b}	68.70 ± 9.94^{c}
CD + 25 ml FW + 25 ml MBS	1185.00 ± 70.53^{ab}	342.25 ± 70.53^{ab}	40.61 ± 8.37^{abc}
CD + 100 ml FW	1196.67 ± 9.46^{ab}	353.92 ± 9.46^{ab}	42.00 ± 1.12^{abc}
CD + 100 ml MBS	1324.17 ± 156.37^{abc}	481.42 ± 156.37^{ab}	57.12 ± 18.55^{abc}
CD + 50 ml FW + 50 ml MBS	1116.67 ± 46.46^{p}	273.92 ± 46.46^{p}	32.50 ± 5.51^{a}
P - Value	0.001***	0.002**	0.004**

[abc] The different superscript letters in a column mean statistically significant difference at p value less than 0.05; *** Very highly significant at p value less than 0.001; ** Highly significant at p value less than 0.01; * Significant at $p < 0.05$; CD: Calcium deficient; SD: Standard calcium diet; FW: Fermented fish waste; MBS: Fermented meat and bone scraps; ROI: Return on investment; PHP: Philippine Peso; The data are expressed as mean ± Standard deviation

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CONCLUSION

This study demonstrated that fermented FW and MBS can serve as alternative calcium sources for quail. Fermented meat and bone scraps provided higher Ca, while both substrates supplied comparable P and Fe. Supplementation with 50 mL MBS/kg produced the better biological response, restoring growth and maximizing hen-day production at 120 days. It improved egg weight, egg mass, shell thickness, and albumen height to levels that matched or exceeded those of the standard diet, with economic performance similarly high. These findings indicated that 50 mL MBS/kg effectively corrected Ca deficiency and offered a sustainable option for quail mineral supplementation. Future study should evaluate the long-term effects of fermented MBS on bone mineralization and eggshell ultrastructure across multiple production cycles, the microbial dynamics and stability of fermented substrates during storage, and the scalability and cost-effectiveness of MBS fermentation under commercial production systems.

DECLARATIONS

Authors' contributions

Rhea Francisco Ramoso and Imelda Ulep Hebron conceptualized and crafted the methodology. Renante Decenella Taylaran and Charly Guillermo Alcantara contributed to data validation and visualization. Nelda Ruba Gonzaga and Eric Randy Reyes Politud contributed to analysis and writing the original draft, and Rudy Mirabueno Camay contributed to data curation and editing. All authors approved the final edition of the manuscript.

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Availability of data and materials

The datasets generated and analyzed in the current study are available upon reasonable request from the corresponding author.

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Competing interests

The authors declared no conflict of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. No AI tools were used to prepare this study.

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