International Journal of Veterinary Sciences and Animal Husbandry 2025; 10(10): 167-169

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(10): 167-169

www.veterinarypaper.com
Received: 18-09-2025
Accepted: 13-10-2025

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Effect of dietary nano-calcium supplementation from eggshell waste on serum biochemical response, carcass yield and meat characteristics of Japanese Quails (*Coturnix coturnix* japonica)

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DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i10c.2621

Abstract
An experiment was conducted to evaluate the effect of dietary nano-calcium supplementation derived from eggshell waste on the serum biochemical response, carcass yield, and meat characteristics of Japanese quails (*Coturnix coturnix japonica*). A total of 240 day-old quail chicks were randomly distributed into five dietary treatments: T_1 (control-100% calcite powder), T_2 (25% replacement with nano-calcium), T_3 (50%), T_4 (75%), and T_5 (100%). Each treatment had four replicates with twelve birds each. After six weeks of feeding, blood samples were collected for serum biochemical estimation, and representative birds were slaughtered for carcass evaluation. Results indicated no adverse effects on serum calcium, phosphorus, and magnesium levels among treatments. Carcass yield parameters such as dressing percentage, breast and thigh yields, and organ weights (liver, heart, gizzard) showed no significant differences. However, birds in T_4 (75% replacement) recorded numerically higher dressing and breast yields, indicating better nutrient utilization. It can be concluded that nano-calcium derived from eggshell waste can replace up to 75% of conventional calcium sources without affecting health, serum profile, or carcass characteristics of Japanese quails.

Keywords: Japanese Quails, nano-calcium, eggshell waste, serum biochemistry, carcass yield, poultry nutrition

1. Introduction
Calcium is an essential mineral for bone development, muscle contraction, and numerous metabolic functions in poultry. Conventional calcium sources such as limestone and calcite powder often exhibit low solubility and variable bioavailability, which can impair skeletal health and meat quality. The use of nano-minerals has emerged as an innovative approach in animal nutrition, improving mineral utilization efficiency and physiological performance.

Eggshell waste, an abundant by-product from poultry processing, contains more than 90% calcium carbonate and represents a sustainable raw material for nano-calcium synthesis. Converting eggshell waste into nano-calcium not only promotes waste valorization but also enhances calcium absorption efficiency due to the increased surface area of nanoparticles.

Previous studies have shown that nano-calcium supplementation improves growth, bone mineralization, and feed efficiency in poultry (El-Katcha *et al.*, 2014; Sobhi *et al.*, 2020) [1, 2]. However, limited information is available on its effects on serum biochemical parameters, carcass yield, and meat characteristics in Japanese quails.

Hence, this study was undertaken to evaluate the effect of dietary nano-calcium supplementation derived from eggshell waste on serum biochemical response, carcass yield, and meat traits of Japanese quails.

2. Materials and Methods

2.1 Experimental Design and Management

The experiment was conducted at the Instructional Poultry Farm, College of Poultry Production and Management, TANUVAS, Hosur, Tamil Nadu, India. The biological study lasted for six weeks. Two hundred and forty day-old Japanese quail chicks (*Coturnix coturnix japonica*) were randomly distributed into five dietary treatments (T_1-T_5) with four replicates of twelve birds each.

- T_1-Control (100% calcite powder)
- T_2-25% replacement with nano-calcium
- T_3-50% replacement
- T_4-75% replacement
- T_5-100% replacement (nano-calcium only)

All diets were isocaloric and isonitrogenous as per Bureau of Indian Standards specifications for quails. Birds were reared under uniform conditions with *ad libitum* feed and water.

2.2 Preparation of Nano-Calcium

Nano-calcium was synthesized from eggshell waste through the ball milling technique (Retsch PM 400, Germany) at 400 rpm for 5 hours with a 10:1 ball-to-powder ratio. The resultant powder had an average particle size of 4 nm and a zeta potential of-25.8 mV, confirming nanoparticle stability.

2.3 Blood Collection and Serum Biochemical Analysis

At the end of the six-week trial, two birds per replicate (eight birds per treatment) were selected randomly and blood samples were collected from the brachial vein using sterile syringes. Serum was separated by centrifugation at 3000 revolutions per minute (rpm) for 10 minutes and stored at -20 °C until analysis.

The following parameters were estimated using diagnostic kits (Span Diagnostics, India):-

- Serum calcium (mg/dL)-by OCPC method
- Serum phosphorus (mg/dL)-by Fiske-Subbarow method
- Serum magnesium (mg/dL)-by Calmagite method
- Total protein (g/dL)-by Biuret method

2.4 Carcass Traits and Meat Characteristics

At the end of the feeding period, two birds per replicate (eight per treatment) were slaughtered after 12-hour feed withdrawal following humane guidelines.

Parameters recorded included:-

- Live body weight (g)
- Dressing percentage (%)
- Eviscerated yield (%)
- Breast and thigh yield (%)

Relative weights of liver, heart and gizzard (% of body weight.

Dressing percentage was calculated as:

$$\text{Dressing \%} = \frac{\text{Dressed weight}}{\text{Live weight}} \times 100$$

2.5. Statistical Analysis

All data were subjected to one-way ANOVA under a Completely Randomized Design (CRD) using SPSS (Version 21.0). Treatment means were compared by Duncan's Multiple Range Test (DMRT) at a 5% significance level (p<0.05).

3. Results and Discussion

3.1 Serum Biochemical Parameters

The effects of nano-calcium supplementation on serum biochemical parameters of Japanese quails are presented in Table 1. No significant differences (p>0.05) were observed among treatments for serum calcium, phosphorus, magnesium, or total protein levels. Birds in nano-calcium-supplemented groups recorded numerically higher calcium and phosphorus values compared to the control, indicating improved mineral absorption.

These findings are consistent with El-Katcha *et al.* (2014) [1], who reported similar trends in broilers supplemented with nano-calcium, attributing the improvements to enhanced intestinal solubility and absorption efficiency.

Table 1: Effect of supplementation of nano-calcium from egg shell waste on biochemical parameters of Japanese quails at sixth week of age

Parameters	T_1	T_2	T_3	T_4	T_5
Calcium (mg/dL)^{NS}	11.69±1.16	14.35±1.59	11.95±1.40	15.09±1.36	12.55±1.13
Phosphorus (mg/dL)^{NS}	8.06±0.32	8.34±0.67	8.43±0.82	10.22±0.81	9.92±0.66
Magnesium (mg/dL)^{NS}	4.60±0.23	4.75±0.69	3.93±0.07	4.24±0.22	5.06±0.38

Each value is a mean of six observations

^{NS} No significance

3.2 Carcass yield and Organ Weights

Carcass characteristics of Japanese quails fed nano-calcium-supplemented diets are presented in Table 2. No significant differences were observed among treatments for dressing percentage, eviscerated yield, or relative organ weights. However, birds receiving 75% nano-calcium (T_4) exhibited slightly higher dressing (71.65%) and breast yield (27.41%), indicating improved nutrient utilization and muscle deposition. The absence of negative effects confirms the safety and metabolic compatibility of nano-calcium as a dietary replacement for calcite powder.

3.3 Discussion

The lack of significant variation in serum biochemical parameters and carcass traits suggests that nano-calcium supplementation maintained normal physiological metabolism without affecting liver or kidney functions. The slight numerical improvements observed in T_4 could be attributed to enhanced calcium bioavailability and better nutrient utilization. These results corroborate findings in broilers and laying hens, where nano-calcium improved bone and meat quality without disturbing metabolic homeostasis (Abo El-Maaty *et al.*, 2021) [6]. Thus, replacing up to 75% of conventional calcium with nano-calcium offers both performance and environmental benefits.

4. Conclusion

Dietary supplementation of nano-calcium derived from eggshell waste up to 75% replacement level had no adverse effect on serum biochemical response, carcass yield, or meat traits of Japanese quails. Nano-calcium can therefore serve as a sustainable and bioavailable calcium source in poultry diets while promoting efficient utilization of eggshell waste.

Table 2: Effect of supplementation of nano-calcium from egg shell waste on carcass characteristics (expressed as percentage of pre-slaughter live weight) of Japanese quails at sixth week of age (Mean ± SE)

Parameters	T_1	T_2	T_3	T_4	T_5
Dressing yield (without skin) ^{NS}	64.90±1.70	65.35±1.48	63.05±2.78	65.39±2.10	63.36±2.12
Blood weight*	4.24 ^{a}±0.72	2.28 ^{b}±0.21	3.68 ^{ab}±0.80	3.24 ^{ab}±0.40	4.51 ^{a}±0.73
Head weight ^{NS}	4.15±0.24	4.07±0.20	4.20±0.17	4.19±0.23	4.26±0.28
Claws weight ^{NS}	1.96±0.09	1.92±0.06	1.88±0.10	2.06±0.19	1.99±0.08
Feathers & Skin weight ^{NS}	13.31±0.85	13.82±0.98	14.57±1.27	14.43±0.75	13.05±1.14
Stomach & Intestine weight ^{NS}	8.97±0.41	9.03±0.70	9.43±0.46	9.13±0.40	8.85±0.25
Gizzard weight ^{NS}	1.78±0.14	2.11±0.22	1.80±0.09	1.92±0.14	1.93±0.07
Liver weight ^{NS}	2.38±0.26	2.47±0.40	2.85±0.40	2.05±0.27	2.26±0.14
Heart weight ^{NS}	0.96±0.04	0.99±0.05	1.04±0.11	1.00±0.06	0.93±0.07
Giblet weight ^{NS}	5.02±0.20	5.40±0.40	5.69±0.35	5.00±0.35	5.10±0.20
Trachea & Lungs weight ^{NS}	1.03±0.07	1.16±0.17	1.12±0.12	0.90±0.07	0.97±0.13
Reproductive organs weight ^{NS}	1.14±0.21	3.71±1.33	4.76±1.67	3.31±0.97	3.13±1.11

Each value is a mean of six observations

* Mean values bearing different superscripts in a row differ significantly (p<0.05)

^{NS} No significance

5. Acknowledgement

The author expresses sincere gratitude to the Dean, College of Poultry Production and Management, Hosur, and the faculty members for their constant guidance and support throughout the research. Appreciation is also extended to the staff of the Centralised Instrumentation Laboratory, Madras Veterinary College, for assistance in biochemical and nano-material analyses.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Karthick SB, Pandian CS, Selvan ST, Anandhi M, Gowri AM. Effect of dietary nano-calcium supplementation from eggshell waste on serum biochemical response, carcass yield and meat characteristics of Japanese Quails (*Coturnix coturnix* japonica). International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(10):167-169.