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Replacement of Shell Grit with Stone Grit and Its Effect on Serum, Carcass and Tibia Bone Characteristics in Egg Type Japanese Quails

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ABSTRACT

A trial was conducted for a period of 46 weeks (6-52 weeks) to evaluate the effect of replacing the shell grit with stone grit in the feed of egg type Japanese quails on serum, carcass and bone characteristics. A total of 360 numbers of five week old female Japanese quails were randomly distributed to 5 dietary treatments with 6 replicates of 12 birds each in a completely randomized design. The calcium in the ration was supplied by replacing shell grit at two different levels (50 and 100 %) with stone grit of two particle sizes (2 mm and 4 mm) and the non replacement (100 % shell grit) group was taken as control. At the end of the 52 weeks, 60 birds were slaughtered to study the carcass and bone parameters. Both right and left tibia bones were collected, defatted and dried. Left tibia bones were measured for length and weight and analyzed for total ash, calcium and phosphorous contents. Right tibia bones were tested for bone breaking strength. Serum samples were analyzed for calcium, phosphorous and alkaline phosphatase. The carcass characteristics such as dressing percentage, eviscerated carcass weight, weights of giblet, liver, heart, gizzard as well as intestinal weight and length, levels of serum calcium, phosphorous and alkaline phosphatase did not vary significantly. The particle size or level of replacement of stone grit did not alter the tibia bone parameters such as tibia weight, length, diameter, Seed or index, Robusticity index and bone breaking strength. From this study, it can be concluded that the stone grit is efficient to supply adequate calcium for growth of bone and muscles as that of shell grit and hence the shell grit can be completely replaced with stone grit of size 4 mm in the feed of egg type Japanese quails without compromising the bone quality and carcass yield.

KEYWORDS: Calcium, Carcass characteristics, Particle size, Stone grit, Tibia bone

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INTRODUCTION

Studies on alternative feed sources have primarily focused on energy and protein rich ingredients. However, limited attention has been given to local alternative sources for major mineral nutrients such as calcium and phosphorus. The main sources of calcium in poultry diets include shell grit, bone meal, limestone, and dicalcium phosphate. Calcium and phosphorus, which are classified as macro-minerals, are essential for birds, as they are key components of bones and play a crucial role in muscle growth. Riberio et al. (2016) experimented in 48 weeks old Japanese quails by feeding with two levels of calcium (29 and 38 g/kg) and two available P levels (1.5 and 3.0 g/kg) for 63 days and found that the supplementation of 38 g Ca/kg (998 mg Ca/bird/day) and 3.0 g avP/kg (78 mg avP/bird/day) increased the marketable egg production and improved the egg quality of Japanese quails at end of the egg production phase.

Coarse limestone particles provide soluble calcium later during the dark period to supply the eggshell formation and reduce the desynchronization between the calcium supply and demand to produce the eggshell. Gloux et al. (2020) concluded that feeding of coarse limestone particles reduced the calcium mobilization from bones which lead to increased bone breaking strength of laying hens. Sinclair-Black (2019) also observed that feeding coarse limestone (1.5 mm) increased blood ionizable calcium concentration in laying hens overnight when more calcium is needed for eggshell formation, in comparison to a fine limestone (0.2 mm)

The most common coarse calcium source used in layer quail diets is shell grit, which contains 36–38% calcium. However, its particle size is irregular and often contaminated with sand and silica. The approximate market price of shell grit ranges from Rs. 8 to 10 per kg. Moreover, collecting shell grit from seashores has become increasingly laborious and expensive. Hence, there is an urgent need to identify an efficient alternative calcium source to replace shell grit. One such alternative is **stone grit,** derived from metamorphosed limestone rocks composed of crystalline forms of calcium carbonate, with a calcium content (38%) equivalent to that of shell grit. It is available in uniform particle sizes with consistent geometric mean diameters and can be used to replace shell grit in layer quail diets. Additionally, stone grit is more affordable (Rs. 4–5 per kg), which may help reduce production costs.

However, there is a lack of research on the use of stone grit in egg type Japanese quails, creating a knowledge gap regarding its impact on serum, carcass and bone characteristics. Determining the ideal particle size and optimum inclusion level of stone grit could help in enhancing the profitability of quail farming. The objective of this study was to evaluate the effects of replacing shell grit with coarse stone grit of two particle sizes (2 mm and 4 mm) at 50% and 100% inclusion levels on serum, carcass and bone characteristics in egg type Japanese quails.

MATERIALS AND METHODS

Location and climate

The biological experiment was conducted in Veterinary College and Research Institute, Namakkal, Tamil Nadu. The temperature humidity index (TSI) during the study period was between 77.52 and 83.07(Anon, 2025).

Ethical and bio-safety committee approval

Biological trial was approved by the Institutional Animal Ethics Committee (IEAC) of Veterinary College and Research Institute, Namakkal (No.16/VCRI/NKL/2024).

Experimental birds

A 52 week biological trial was conducted by using 360 numbers of five week old "*TANUVAS Namakkal Gold Quail,"* developed for egg production were wing banded, weighed, and randomly assigned to five treatment groups with six replicates of 12 birds each in a completely randomized design. All birds were reared in cage system and fed an isocaloric and isonitrogenous diet with 3% calcium and 0.40% non-phytate phosphorus. The dietary calcium of 3% was provided to dietary treatments as follows.T1 -100 % shell grit (control),T2 -100 % stone grit with 2 mm size, T3 -100 % stone grit with 4 mm size, T4 -50 % shell grit plus 50 % stone grit with 2 mm size, T5 -50 % shell grit plus 50 % stone grit with 4 mm size. Calcium was supplied by replacing shell grit with stone grit (2 mm and 4 mm) at 50% and 100% levels. Maize and soya based experimental diets were identical in nutrient composition, differing only in calcium source, level, and particle size. The in vitro solubility of 2 mm and 4 mm stone grit was estimated as 61.9 and 54.2 per cent respectively (Premkumar et al., 2024)

Blood collection and serum separation

At the end of experimental period, three ml of blood was collected (jugular vein) from twelve birds in each treatment group during slaughter. Blood samples were collected in test tubes and allowed to clot by keeping in slant position and then centrifuged for 10 minutes at 2000 rpm in a centrifuge to separate clear supernatant serum. The serum samples were stored in the deep freezer at -20^{0}C until further analysis.

Serum calcium, phosphorous and alkaline phosphatase estimation

The pooled serum samples for each replicate were analyzed in M/S Biosystems A50 India auto analyzer available in the Clinical Laboratory facility located in the Clinical complex of Veterinary College and Research Institute, Namakkal. The serum calcium, phosphorous and alkaline phosphatase were estimated by using the specific commercial readymade kits purchased from Dhaksha Diagnostics, Salem, India. The values generated in the auto analyser for serum calcium and phosphorous were measured as mg/dL and the serum alkaline phosphatase was measured as U/L.

Carcass characteristics assessment

At the end of 52 weeks of experimental period, two birds having the body weight nearer to the mean body weight were selected from each replicate in all the treatment groups and feed was withdrawn 8 hours prior to slaughter and potable water was provided *adlibitum*. Birds were weighed before slaughter and humanely sacrificed (Genchev and Mihaylo, 2008). Blood was collected for serum separation and stored frozen. Carcasses were manually defeathered, eviscerated, and organs (heart, liver, gizzard without contents, and intestine) were weighed. Pre-slaughter, post-bleeding, defeathered, and eviscerated weights, along with organ and giblet weights, were recorded and expressed as percentage of live weight. Intestinal length was measured with a measuring tape, and both left and right tibia were collected and stored at −20°C for further analysis.

Bone quality assessment

1. Collection of tibia bone

At the end of the 52weeks, both left and right tibia bones were dissected from the slaughtered birds and their adhering muscles along with connective tissue were removed manually. The collected bones were processed by dipping in 10 per cent sodium hydroxide solution for five minutes to remove the adhering fine and soft tissues. De-fattening of dried bones was done by dipping in petroleum ether for overnight following the procedure of AOAC, (2023) and then the bones were dried in a hot air oven at 100°C for 12 hours.

2. Tibia weight and length measurement

Bone weight was measured using a digital electronic scale to the nearest 0.001g in fresh and fat-free dried tibia bone. The length of the tibial bone was measured in digial vernier caliper (Mitutayo Make) from the proximal end to the distal end of the bone. The circumference was measured at their mid points.

3. Estimation of tibial ash, calcium and phosphorous

Tibial ash is the non combustible portion representing the total mineral matter content of the bone. The determination of crude ash was performed as per the standard procedure (Reference IS 14827:2000) in the Animal Feed Analytical and Quality Assurance Laboratory, VCRI, Namakkal. The dry defatted tibia bones were weighed and burnt into ash in a muffle furnace at 600°±30° C for 12 hours as per the procedure described by Panda et al. (2006) and the percentage of total ash was calculated and then cooled in a dessicator to room temperature. The percentage of total ash was calculated by the following formula.

$$\text{Total ash per cent} = \frac{\text{Weight of ash in the crucible (g)}}{\text{Initial weight of the bone sample (g)}} \times 100$$

4. Estimation of tibial calcium

Tibial calcium per cent was estimated from the tibial ash by the titration method as defined by AOAC (2023) official method 927.02 (Dry ash method for calcium estimation in animal feed). The calcium in the sample was precipitated as calcium oxalate by using ammonium oxalate in acidic medium. The precipitated calcium oxalate was filtered out, washed with ammonium hydroxide to free ammonium oxalate from the precipitate and dissolved in hot sulphuric acid and the liberated oxalic acid was estimated by permanganometric titration.

1ml of 0.1N $KMNO_4$ = 0.002 g of calcium

5. Estimation of tibial phosphorous

Tibial phosphorous per cent was estimated from the tibial ash by the standard calorimetric method as defined by AOAC (2023) official method 965.17 (Dry ash method for phosphorous estimation in animal feed). Phosphorous in the sample is converted into phosphomolybdo vanadate complex by adding the ammonium molybdo vanadate reagent. The intensity of colour of the phosphomolybdo vanadate complex was measured at 400 nm in UV visible spectrophotometer.

6. Bone mineral density

The seedor index (g/cm) was calculated by dividing the tibial bone dry weight by its length, as an indicative of bone mineral density (Seedor et al., 1991). Robusticity index and Seedor index were calculated by the following equations (Mohammed et al., 2021).

Seedor index = Weight of the tibia in g / Length of tibia in cm × 100

Robusticity index = Tibia length in cm /cube root of bone weight in g.

Statistical Methodology

The biological trial data collected on carcass, bone and serum parameters were statistically analyzed by one way ANOVA method in SPSS 20th version and the mean of different experimental groups were tested for statistical significance by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of particle size and replacement level of stone grit on serum profile in egg type Japanese quails

1. Serum calcium

The mean serum calcium (mg/dL) of egg type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 1. The calcium source, particle size and replacement levels of stone grit in this study did not significantly (P≥0.05) affect the serum calcium of 52 week old Japanese quails. Similar non significant serum calcium levels with respect to particle size of calcium source were reported in layers, (Nisar 2015; Saki et al., 2018; Lee et al., 2021) and in broilers (Hu et al., 2022). The findings of this study are consistent with those of Novack et al. (2023) who replaced limestone with industrial egg residue in broilers or with cuttlefish bone in the diet of Japanese quails (Bugdayci et al., 2020).

In contrast, Ertas et al. (2006) found highest plasma calcium level in Japanese quails when limestone was replaced with macunim shell at 75 per cent (28.2 mg/dl) as compared to the control group (23.5 mg/dl). Similarly, replacement of limestone with 30 percent coated calcium (Dongare et al., 2024) significantly (P<0.05) increased the serum calcium level, whereas, replacement of limestone with eggshell at 100 per cent (Karim and Abdulla, 2024) in broiler diet resulted significantly (P<0.01) lower serum calcium level as compared to the control group (8.78 vs 13.32 mg/dL).

The absence of significant differences in serum calcium levels in the present study suggests that replacing shell grit with stone grit (2 mm or 4 mm) at 50 or 100 per cent level in the diet of Japanese quail does not impair calcium homeostasis. These results imply that as long as the overall dietary calcium content is adequate, serum calcium levels can be maintained within optimal physiological limits, regardless of calcium source or particle size.

Table 1. Mean (±S.E.) of serum calcium, phosphorous and alkaline phosphatase of egg type Japanese quails fed diet with different particle sizes and replacement levels of stone grit

Serum profile parameters	T1	T2	T3	T4	T5	F-Value	P-Value
Serum calcium (mg/dL)	12.60 ± 0.67	11.37 ± 1.33	10.40 ± 0.37	9.95 ± 0.40	11.85 ± 0.96	1.68 NS	0.187
Serum phosphorus (mg/dL)	7.32 ± 0.55	6.73 ± 0.64	5.18 ± 0.50	6.20 ± 0.20	6.53 ± 0.72	2.06 NS	0.117
Serum alkaline phosphatase (U/L)	742.7 ± 82.5	631.6 ± 99.3	535.6 ± 36.3	764.7 ± 57.1	584.8 ± 59.6	1.99NS	0.128

Each value is the mean of 12 observations. Values in each row having a common superscript do not differ significantly.
*(P<0.05) ** (P0.01) NS (P>0.05)
T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit
mg/dL- miiligram per deciliter, U/L – Units per litre, mm- millimeter
S.E – Standard error

2. Serum phosphorous

The mean serum phosphorus levels (mg/dL) of egg-type Japanese quails fed diets with different sizes and replacement levels of stone grit are presented in Table 1. The results of this study revealed that serum phosphorus was not significantly (P≥0.05) influenced by the calcium source, particle size, or level of replacement of stone grit in 52-week-old Japanese quails. These findings are consistent with those of Bugdayci et al. (2020), who reported no significant changes in serum phosphorus levels in 8-week-old Japanese quails when limestone was replaced with cuttlefish bone at 0, 50, and 100 per cent levels. A similar observation was made by Karim and Abdulla (2024), who found no significant differences when limestone was replaced with eggshell at 50 and 100 per cent levels in broiler diets. Likewise, non-significant variations in serum phosphorus concentrations with respect to calcium particle size have been reported in layers (Rao and Raju, 2004; Gultepe et al., 2021) and broilers (Hu et al., 2022). In contrast, Souvik-Mondal et al. (2011) recorded significantly higher (P<0.05) plasma phosphorus levels in 3-week-old Japanese quails fed diets containing tricalcium phosphate and dicalcium phosphate compared to other calcium sources. Similarly, Dongare et al. (2024) observed a significant increase in serum phosphorus (P<0.01) in broilers fed diets with 30 per cent coated calcium (5.57 mg/dL) compared to the control (4.90 mg/dL). The results of the present study indicate that replacing shell grit with stone grit (2 mm or 4 mm) at 50 or 100 per cent levels does not alter phosphorus metabolism, provided that the dietary calcium-to-phosphorus ratio is adequately maintained.

3. Serum alkaline phosphatase

The mean serum alkaline phosphatase (U/L) levels of egg-type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 1. Serum alkaline phosphatase activity was not significantly (P≥0.05) influenced by the calcium source, particle size, or level of replacement of stone grit in 52-week-old Japanese quails. These findings are consistent with those of Hu et al. (2022), who reported no significant effects of calcium level or limestone particle size on alkaline phosphatase activity in broilers.

Physiologically, elevated alkaline phosphatase activity is associated with osteoblastic activity, which is more pronounced during periods of growth and bone remodelling. The relatively lower enzyme levels observed in the present study may be attributed to the advanced age of the birds (52 weeks), aligning with the observations of Brenes et al. (2003), who reported a decline in alkaline phosphatase activity with age. Nevertheless, the numerically higher alkaline phosphatase values observed in the control group (T1) and the 50 per cent 2 mm stone grit replacement group (T4) may reflect ongoing bone remodelling processes. The absence of significant differences in serum alkaline phosphatase levels among treatments indicates that replacing shell grit with stone grit (2 mm or 4 mm) at 50 or 100 per cent levels in the diet of Japanese quails maintains phosphorus homeostasis, provided that the available phosphorus concentration is maintained at 0.40 per cent in both control and treatment diets.

Effect of particle size and replacement level of stone grit on carcass characteristics of egg type Japanese quails

1. Dressing per cent

The mean dressing per cent of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit at 52 weeks of age is presented in Table 2. The results revealed that dressing per cent of Japanese quails fed with different sizes and replacement levels of stone grit at 52 weeks of age did not differ significantly (P>0.05). Similar non significant effects on dressing per cent was observed in Japanese quails supplemented with calcium propionate at the rate of 6g/kg in the diet (Bonos et al., 2010) and in broilers fed with granite grit (Garipoglu et al., 2006), marble powder (Demirel et al., 2017) and various calcium sources like oyster shell, eggshell, and calcium carbonate powder (Rezvani et al., 2019). In this study, grit particle size (2 mm vs. 4 mm) also had no significant impact, consistent with findings by Moghaddam et al. (2016), who observed no difference in dressing percentage when broilers were fed insoluble grit of 2, 3, and 4 mm sizes.

However, contrasting results were noted by Maranan et al. (2021), who found a linear decline in dressing percentage with increasing dietary eggshell levels. Moreover, Hakami et al. (2022) also found significantly higher dressing per cent in broilers fed marine mineral complex (CeltiCal) at 0.4 per cent level as a partial substitute for limestone in broilers compared to the control. As the dressing per cent is indicative of adequate flesh and fat cover of the carcass, it can be inferred that replacement of shell grit with stone grit in the feed supplied sufficient calcium ions for uniform growth of body tissues, organs, skin and feathers irrespective of the particle size.

2. Ready to cook weight

The mean ready to cook weight of egg type Japanese quails fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The results revealed that ready to cook weight of Japanese quails fed with different sizes and replacement levels of stone grit at 52 weeks of age did not differ significantly (P>0.05).

These results are in accordance with the findings of Mendonca et al. (2022) who conducted a trial in European quails fed with five different calcium sources (calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal or oyster shell meal) from day old to 35 days of age and found no significant difference (P>0.05) in absolute and relative weight of carcass. Similarly, Novack et al. (2023) reported no significant differences in carcass yield percentages in broilers by replacing limestone with industrial egg residue at 0, 35, 70, and 100 per cent.

In contrast, Idachaba (2013) reported significant differences in dressed body weights of broilers fed diet with different levels of stone grit in rice offal, and by Usaro and Christopher (2022), who reported improved dressed weights in broilers fed various levels of insoluble granite grit in a brewer's spent grain-based diet. The improved dressed weights were attributed to enhanced nutrient utilization due to the inclusion of grit in the diet. However, in the present study, inclusion of stone grit either 2 or 4 mm at both 50 and 100 per cent level in the diet did not exhibit additional advantage in terms of more dressed weight as compared with control diet.

3. Eviscerated carcass yield

The mean eviscerated carcass yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The results revealed that eviscerated carcass yield of Japanese quails fed with varying stone grit sizes and replacement levels at 52 weeks of age did not differ significantly (P>0.05).

These findings align with Moghaddam et al. (2005), Garipoglu et al. (2006), and Eser et al. (2018), who also reported no significant effects on carcass yield following the inclusion of zeolite or granite grit in broiler diets. Likewise, Mendonca et al. (2022) found no significant differences in carcass yield when European quails were fed five different calcium sources (calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, or oyster shell meal) from day old to 35 days of age. However, contrasting results were reported by Dongare et al. (2024) and El-Ganainy et al. (2024) who reported significantly higher carcass yield in broilers supplemented with coated calcium and nano hydroxyapatite, respectively. These results suggest that while certain advanced calcium forms may influence carcass yield, traditional sources like shell grit and stone grit do not significantly impact eviscerated carcass yield in Japanese quail.

4. Giblet yield

The mean giblet yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The giblet yield (per cent of live body weight) was not significantly (P≥0.05) influenced by both particle size and level of replacement of stone grit in 52 weeks old Japanese quails. This finding is consistent with Gongruttananun (2011), who reported no significant differences in giblet weights when ground eggshell replaced limestone in breeder male diets. In contrast, Dongare et al. (2024) and El-Ganainy et al. (2024) observed significantly higher giblet weights in broilers fed coated calcium and nano-hydroxyapatite, respectively. Overall, the present study suggests that giblet yield in Japanese quails is not influenced by the calcium source or its particle size.

5. Liver yield

The mean liver yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The liver yield (per cent of live body weight) was not significantly (P≥0.05) influenced by either the particle size or the replacement level of stone grit at 52 week old Japanese quails. These results are consistent with the findings of Moghaddam et al. (2016) who fed different sizes of stone grits; Demirel et al. (2017) who fed different levels of marble powder and Eser et al. (2018) who fed granite grit in broilers and found no statistical difference in liver yield among the treatment groups. The current findings are further supported by previous studies conducted by Hakami et al. (2022) in broilers, Mendonca et al. (2022) in European quails and Rezende et al. (2024) in Japanese quails, all of which confirmed that the source of calcium did not influence the relative weight of the liver. Similarly, Pacheco *et al.* (2022) and Lima et al. (2024) observed that the particle size of limestone in layer diets did not significantly affect relative liver weights. The present results revealed that relative liver weight is independent of the particle size of stone grit and its inclusion level.

Table 2. Mean (±S.E.) carcass characteristics of egg type Japanese quails fed diet with different particle sizes and replacement levels of stone grit

Carcass Characteristics	T1	T2	T3	T4	T5	F-Value	P-Value
Pre slaughter weight (g)	264.7±4.8	260.0±7.6	263.3±3.9	260.4±3.2	257.7±3.3	0.33 NS	0.855
Ready-to-cook body weight(g)	171.05±4.77	170.46±5.3	170.80±4.24	169.04±2.58	166.80±2.96	0.18 NS	0.945
Eviscerated carcass yield (%)	59.18±0.80	60.46±0.92	59.59±0.91	59.52±0.66	59.75±0.61	0.36 NS	0.837
Dressing percentage	64.53±0.88	65.61±0.95	64.79±0.90	64.93±0.68	64.70±0.60	0.27 NS	0.898
Liver yield (%)	2.68±0.17	2.43±0.15	2.54±0.12	2.65±0.21	2.39±0.15	0.56 NS	0.694
Gizzard yield (%)	1.85±0.09	1.90±0.14	1.85±0.05	1.87±0.07	1.82±0.09	0.09 NS	0.984
Heart yield (%)	0.810±0.04	0.815±0.05	0.810±0.04	0.900±0.04	0.753±0.03	1.75 NS	0.151
Giblet yield (%)	5.35±0.14	5.15±0.22	5.20±0.12	5.41±0.24	4.96±0.18	0.92 NS	0.456
Relative intestinal weight (%)	4.64±0.12	4.85±0.24	4.57±0.19	4.55±0.14	4.61±0.16	0.43 NS	0.795
Relative intestinal length (cm)	29.55±0.63	29.54±1.24	28.06±0.82	27.97±1.11	28.28±0.90	0.69NS	0.603

Each value is the mean of 12 observations. Values in each row having common superscript do not differ significantly * (P<0.05) ** (P<0.01) NS (P>0.05)

T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit

S.E – Standard error, mm- millimeter, cm- centimeter, g- grams

6. Heart yield

The mean heart yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The heart yield (per cent of live body weight) was not significantly influenced by either particle size or level of replacement of stone grit in 52 week old Japanese quails. These results are supported by Mendonca et al. (2022) who reported no significant difference (P>0.05) in absolute and relative heart weight when non sexed European quails were fed five different calcium sources (calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, or oyster shell meal) from day old to 35 days of age. Similarly, Hakami et al. (2022), Demirel et al. (2017), and Moghaddam et al. (2016), who also reported no significant effect of various calcium sources or grit types on heart weight in broilers. In contrast, Bonos et al. (2010) found that calcium propionate significantly reduced heart weight, while Dongare et al. (2024) reported increased heart weight in broilers with inclusion of coated calcium.

Since heart size is indirectly indicative of cardiac output capacity, birds with larger heart may better meet high metabolic demands. In this study, the absence of significant differences in heart weight suggests that variations in calcium source and particle size did not alter cardiac output capacity in Japanese quails

7. Gizzard yield

The mean gizzard yield of egg type Japanese quail fed diet with different sizes and replacement levels of stone grit is presented in Table 2. The gizzard yield (per cent of live body weight) was not significantly (P≥0.05) influenced by either the particle size or level of replacement of stone grit in 52 week old Japanese quail. These results are consistent with studies by Pacheco et al. (2022), who found no significant effect of calcium particle size on gizzard yield in layers. Similarly, Rezvani et al. (2019), Hakami et al. (2022) reported no significant changes in gizzard weight with various calcium sources in broilers. Moreover, no significant differences in relative and absolute gizzard weights were found when European quails fed calcitic lime, calcium carbonate, charru mussel shell meal, macunim shell meal, or oyster shell meal (Mendonca et al., 2022) and when Japanese quails fed with 0.5 percent calcareous seaweed types 1 and 2 as partial replacements for limestone (Rezende et al., 2024). In contrast, Aroujo et al. (2011) reported that coarse limestone (1.00 mm) significantly (P<0.01) increased gizzard yield compared to fine limestone (0.60 mm) in layers.

Gizzard weight reflects muscle development and the thickness of the muscle tunic. Grit size affects gizzard stimulation, as harder and larger particles stay longer in the gizzard compared to softer or smaller particles, promoting greater muscular development (Gionfriddo and Best, 1999). Eser et al. (2018) reported that inclusion of insoluble grit in broiler diets increased relative gizzard weight and reduced abdominal fat, suggesting improved digestive function and leaner body composition. However, in the present study, feeding stone grit (2 mm or 4 mm) did not significantly increase gizzard weight, indicating that the soluble stone grit used was not as effective in grinding feed particles as insoluble grits, and therefore were insufficient to induce notable changes in gizzard development.

8. Relative intestinal weight

The mean relative intestinal weight of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 2. The relative intestine weight (per cent of live body weight) was not significantly (P≥0.05) influenced by either the particle size or replacement level of stone grit in 52 week old Japanese quails.

The present findings align with Rezvani et al. (2019), who reported no significant differences in intestinal weight of broilers fed various calcium sources, including calcium carbonate, oyster shell, and eggshell powder. Similar results were observed in layers by Pacheco et al. (2022) and Lima et al. (2024) who found no effect of fine or coarse limestone particles on relative intestinal weight. In contrast, El-Ganainy et al. (2024) reported higher intestinal weight in broilers fed 100 per cent dicalcium phosphate, and Hakami et al. (2022) noted lower intestinal weights in broilers not supplemented with marine calcium source. Overall, the current study suggests that calcium source and grit particle size do not significantly affect intestinal weight in Japanese quails.

9. Relative intestinal length

The mean relative intestinal length of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 2. The relative intestine length (per cent of live body weight) was not significantly (P≥0.05) influenced by both particle size and level of replacement of stone grit in 52 week old Japanese quails.

These findings are consistent with Aroujo et al. (2011) and Pacheco et al. (2022) who also found no significant impact of calcium level or particle size on intestinal length in layers. In contrast, Garipoglu et al. *(*2006) reported increased gut length in broilers fed granite grit, and El-Ganainy et al. (2024) found longer intestines in broilers fed dicalcium phosphate compared to nano-hydroxyapatite. Overall, the present study suggests that calcium source and grit particle size do not influence intestinal length in egg type Japanese quails.

Effect of particle size and replacement level of stone grit on bone characteristics of egg type Japanese quail

1. Tibia weight, length and diameter

The mean tibia weight, length and diameter of egg type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 3. The tibia dry weight, length, and diameter were not significantly (P≥0.05) influenced by either the particle size or the level of stone grit replacement in 52 week old Japanese quails.

These results are consistent with studies by Codeiro et al. (2017), Lee et al. (2021) and Pacheco et al. (2022) who found no significant effect of calcium source or particle size on tibia parameters in laying hens. Conversely, De witt et al. (2009) found that large limestone particles (2-3.8 mm) in the diet of laying hens resulted in significantly decreased tibia length (P=0.0317) and tibia weight (P=0.0265) at 37 weeks of age when compared with small (0-1.0 mm) and medium (1.0-2.0 mm) limestone particles. Limestone particle size also affected the absolute femur and tibia weight at an earlier age (4 weeks) where pullets fed coarse limestone which had significantly heavier femur (P=0.011) and tibia (P=0.008) than pullets fed fine or mixture of fine and coarse limestone (Khanal et al., 2020). A similar trend was observed by Poudel et al. (2022) who found significantly higher tibia length in 15:85 FL: CL under conventional feeding and 0:100, 35:65 FL: CL groups under split feeding systems. Hakami et al. (2022) substituted limestone with marine calcium sources at different levels in broiler diets and found no significant differences in tibia and femur length or width, although tibia weight was significantly higher in marine calcium supplemented groups compared to the control. A comparable result was reported by Karim and Abdulla (2024) who replaced limestone with eggshell at 50 and 100 per cent levels in broiler diets, while tibial length was not significantly different among the groups, tibial weight was significantly higher (P=0.008) in the group fed 100 per cent eggshell compared to the other two groups. Overall, the results suggest that stone grit can effectively replace shell grit up to 100 per cent without negatively impacting tibia growth in egg type Japanese quails at 52 weeks of age.

2. Tibia ash

The mean tibia ash (per cent) on dry matter basis of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 3. The tibia ash (percent) was not significantly (P≥0.05) influenced by either the particle size or level of replacement of stone grit in 52 week old Japanese quails. These findings align with studies by Bueno et al. (2016), Codeiro et al. (2017) and Lima et al. (2024) who reported no significant impact of calcium particle size or source on tibia ash in older hens and broiler breeders.

Contrary observations of higher tibia ash by feeding large sized calcium source were observed in broilers (Manangi and Coon, 2007), layers (De witt et al., 2009; Xavier et al., 2015; Mannangi et al., 2018) and layer chicks (Khanal et al., 2020). But, Pacheco et al. (2022) found that fine grained limestone resulted in better mineral matter in the tibia when 3.8 per cent of dietary calcium is provided. In contrast to the present findings, Hakami et al. (2022) and Dongare et al. (2024) found significantly higher tibia ash in broilers when limestone was partially replaced by marine calcium source or with coated calcium compared to the control group. Maranan et al. (2021) also found a linear increase in tibia ash content when limestone was replaced with eggshell powder (0, 50, 75, and 100 per cent) in broilers. These findings suggest that both limestone and eggshell can contribute to bone mineralization in varying proportions depending on inclusion levels. The results of this study inferred that stone grit can be included in the diet of egg type Japanese quails with replacement of shell grit either at 50 or 100 per cent level without compromising the bone development and mineralization at the age of 52 weeks.

3. Tibia calcium

The mean tibia calcium content (per cent) on dry matter basis of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit is presented in Table 3. Tibia calcium percent was not significantly ($P \geq 0.05$) influenced by either the particle size or the level of replacement of stone grit in 52 week old Japanese quails. The particle size, calcium source and replacement levels in this study did not significantly influence the tibia calcium per cent.

These results agree with Bueno et al. (2016), who found no effect of limestone particle size on tibia calcium in aged broiler breeders. Similarly, Moura et al. (2020) also reported no significant changes in tibial calcium when limestone was replaced with quail eggshell powder in Japanese quails. In contrast, Molnar et al. (2017) reported lower tibia calcium in hens fed 100 per cent coarse limestone under conventional feeding. Higher tibia calcium was also observed in broilers and layers fed coarse calcium sources (Bassi et al., 2022 and Pacheco et al., 2022). Maranan et al. (2021) noted a quadratic response in broilers, with tibia calcium increasing up to 50 per cent eggshell replacement and then declining. Similarly, Karim and Abdulla (2024) found significantly higher tibial calcium in birds fed 0 per cent eggshell, followed by those on 100 per cent eggshell diets. From this present study, it can be inferred that partial or full replacement of shell grit with stone grit did not affect tibia calcium levels in Japanese quails at 52 weeks, indicating that stone grit is a viable alternative calcium source for maintaining bone mineralization.

4. Tibia Phosphorous

The mean tibia phosphorous per cent of egg type Japanese quails fed diet with different sizes and replacement levels of stone grit are presented in Table 3. Tibia phosphorous (percent) was not significantly ($P \geq 0.05$) influenced by either the particle size or level of replacement of stone grit in 52 week old Japanese quails. The particle size, calcium source and replacement levels in the study did not significantly influence the tibia phosphorous per cent. These results align with studies by Bueno et al. (2016) and Wang et al. (2014) who found that calcium source and particle size did not significantly impact tibia phosphorus in broiler breeders and laying ducks, respectively.

However, Bassi et al. (2022) reported lower tibia phosphorus in broilers fed coarse oyster shell meal with a high calcium/ phosphorous ratio, while Jafari-Arvari et al. (2024) observed higher phosphorus in broilers fed coarse limestone. Similarly, Karim and Abdulla (2024) found significantly higher ($P=0.001$) tibial phosphorus content in birds fed diets with 0 per cent eggshell, followed by 100 per cent eggshell in broiler diets. Ajani et al. (2024) also noted significant variation in tibia phosphorus based on the inclusion of bone dust in layer diets. These findings suggest that different calcium sources can contribute to bone mineralization in varying proportions depending on their inclusion levels.

Phosphorus content in the tibia is a strong indicator of bone resorption status. The tibia serves as a responsive calcium reservoir for eggshell formation, particularly during the dark period when feed intake is minimal. Inadequate dietary calcium can create a competition between maintaining structural bone strength and supporting eggshell formation, leading to mobilization of calcium from both structural and medullary bone to the uterus (Clunies et al., 1992). During this process, phosphorus is also released due to breakdown of hydroxyapatite, potentially resulting in phosphatemia if bone resorption becomes excessive. The lack of variation in tibia phosphorus content among treatments in the present study clearly indicates that bone remodeling in response to calcium demands for eggshell production occurred efficiently, without compromising bone mineral status, regardless of calcium source, particle size, or inclusion level.

5. Tibial mineral density

The mean Seedor index and Robusticity index of egg type Japanese quails fed diets with different particle sizes and replacement levels of stone grit are presented in Table 3. The Seedor index and Robusticity index were not significantly ($P \geq 0.05$) influenced by calcium source, particle size and level of replacement of stone grit in 52 week old Japanese quails. These findings align with Rezende et al. (2024) and Moura et al. (2020) who also reported no significant changes in bone density indices when replacing limestone with calcareous algae or quail eggshell powder in the diet of broilers or Japanese quails. Similar non-significant effects of particle size on Seedor index were reported by Wang et al. (2014), Codeiro et al. (2017) and Pacheco et al. (2022).

However, Lee et al. (2021) found that hens fed eggshell fine particles and oyster shell had significantly higher tibia bone mineral density than those fed limestone, cockle shell, and eggshell coarse particles over a 7 week experimental period ($P<0.001$). Conversely, Eusebio-Balcazar et al.

(2018) found that feeding a blend of coarse and fine limestone (average particle size 0.875 mm) to Lohmann Brown and Bovans White pullets improved bone mineralization and bone mineral density (P=0.034) compared to feeding only fine limestone (0.431 mm). Additionally, other researchers have observed that feeding finer calcium sources led to significantly higher bone mineral density in layer chicks (Khanal et al., 2020) and layers (Lima et al*.,* 2024). The lack of variation in the bone density indices among the treatments in this study clearly inferred that remodeling of bones in response to the calcium demand for eggshell production takes place promptly without compromising the bone integrity irrespective of the calcium sources particle size and inclusion level.

6. Tibia bone breaking strength

The mean tibia bone breaking strength of egg type Japanese quails fed diets with different sizes and replacement levels of stone grit are presented in Table 3. The calcium source, particle size and replacement levels of stone grit in this study did not significantly (P≥0.05) affect the tibia bone breaking strength of 52 week old Japanese quails. Similarly, inclusion of different sizes of limestone particles in the diet did not significantly affect (P>0.05) tibial breaking strength in 82 week old post moulting broiler breeders (Bueno et al*.,* 2016). Likewise, Lee et al. (2021) and Mendonca et al. 2022 found that tibia breaking strength did not differ among the dietary groups fed with different calcium sources in laying hens and European quails (P>0.05). Wang et al. (2014) fed laying ducks with limestone of two particle sizes (<0.1 mm; 0.85 to 2 mm), and Lima et al. (2024) who fed layers with limestone of two particle sizes (fine 0.222 mm and coarse with 3.332 mm) and found no significant changes in the tibia breaking strength.

In contrast, Leao et al. (2020) found higher bone tensile strength in the European quails fed diets containing 0.684 per cent calcium derived from charru mussel shell meal than from calcitic limestone, calcium carbonate, macunim shell meal and oyster shell meal. On deviation with the present results, Xavier et al. (2015) and Karim and Abdulla (2024) found that increasing levels of coarser limestone or eggshell resulted in a linear increasing effect on the bone breaking strength of the tibia of laying hens and broilers. Whereas, feeding finer calcium sources resulted in significantly greater bone breaking strength in layer chicks (Khanal et al., 2020) and layers (Poudel et al., 2022). The non variation in the bone breaking strength among the treatments in this study clearly inferred that remodeling of bones in response to calcium demand for eggshell formation takes place promptly without altering the morphometric characteristics of the bone irrespective of calcium sources, particle sizes and inclusion levels.

Effect of particle size and replacement level of stone grit on cost economics of egg type Japanese quail

The mean feed cost per kg egg mass or dozen egg, cost of production per kg egg mass or dozen egg was highest in T5 and lowest in T3 and intermediate in other treatment groups, however the differences were not statistically significant (P≥0.05). The benefit- cost ratio was highest in T3 and lowest in T5 and intermediate in other treatment groups, however the differences were not statistically significant (p≥0.05). The higher benefit- cost ratio in T3 may be due to better feed efficiency and livability over the other treatment groups. In a related study, Inoti (2020) conducted an experiment on laying hens using two types of limestone, one sourced from the Athi River near Nairobi (AR) and the other from Ukunda in the coastal region of Kenya (UKC). These sources differed in particle size distribution and *in-vitro* solubility. The study found that the feed cost was lower in birds that consumed UKC limestone compared to those fed AR limestone for producing one kg of egg mass, highlighting the role of calcium source. In contrast, Moura et al. (2020) substituted limestone with quail eggshell powder at 0, 25, 50, 75 and 100 per cent in Japanese quails and found better economic efficiency index (P>0.05) at 25 per cent and 50 per cent level as compared to other treatment groups. However, Islam and Nishibori (2021) reported lowest production cost in hens fed 8 per cent eggshell, followed by 8 per cent limestone, 8 per cent oyster shell, 4 per cent eggshell, 4 per cent limestone and 4 per cent oyster shell, highlighting the role of calcium source. The replacement of shell grit with 2 or 4 mm stone grit at 100 and 50 per cent levels in the diet of Japanese quails did not significantly (P≥0.05) improve the economical parameters such as feed cost per kg egg mass, production cost per kg egg mass, feed cost per dozen egg, production cost per dozen egg, production cost per egg and benefit-cost ratio when compared to control group. This may be due to the insignificant differences in feed efficiency and livability among all treatment groups throughout experimental period of 6 to 52 weeks of age.

Table 3.Mean (±S.E.) tibia bone characteristics of egg type Japanese quails fed diet with different sizes and replacement levels of stone grit

Tibia bone characteristics	T1	T2	T3	T4	T5	F-Value	P-Value
Ready-to-cook body weight(g)	171.05±4.77	170.46±5.3	170.80±4.24	169.04±2.58	166.80±2.96	0.18 NS	0.945
Weight (dry) (g)	0.621± 0.02	10.697 ±0.034	0.612 ± 0.032	0.666 ± 0.049	0.662 ± 0.045	0.86 NS	0.495
Length (cm)	5.41 ± 0.11	5.50 ± 0.05	5.30 ± 0.14	5.39 ± 0.04	5.34 ± 0.07	0.79 NS	0.539
Diameter (cm)	0.283 ± 0.005	0.287 ± 0.006	0.282 ± 0.008	0.277 ± 0.003	0.279 ± 0.002	0.55 NS	0.702
Total ash (%)	56.51 ± 1.05	59.91 ± 0.82	59.22 ± 1.39	58.98 ± 1.19	60.47 ± 1.51	1.54 NS	0.221
Calcium (%)	21.21 ± 0.88	22.70 ± 0.51	22.08 ± 0.77	22.07 ± 0.56	21.72 ± 1.04	0.51 NS	0.728
Phosphorus (%)	10.62 ± 0.20	11.27 ± 0.12	10.96 ± 0.26	10.93 ± 0.22	11.29 ± 0.24	1.74 NS	0.172
Seedor index (g/cm)	11.53 ± 0.44	12.64 ± 0.57	11.58 ± 0.56	12.31 ± 0.80	12.35 ± 0.71	0.64 NS	0.634
Robusticity index	6.36 ± 0.13	6.24 ± 0.08	6.28 ± 0.17	6.23 ± 0.10	6.17± 0.09	0.33 NS	0.853
Breaking strength (N)	16.35 ± 0.68	16.69 ± 0.97	17.50 ± 0.83	18.63 ± 0.78	18.55 ± 0.80	1.62 NS	0.183

Each value is the mean of 12 observations. Values in each row having a common superscript do not differ significantly.

*(P<0.05) ** (P<0.01) NS (P>0.05) S.E – Standard error, mm- millimeter, cm- centimeter, g/cm- gram per centimeter, N- Newton

T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit

Table 4. Mean (± S.E.) cost economic parameters of egg type Japanese quails fed diet with different particle sizes and replacement levels of stone grit

Cost economic parameters	T1	T2	T3	T4	T5	F-Value	P-Value
Feed cost / kg egg mass (Rs.)	129.55 ± 2.77	133.21 ± 3.30	124.72 ± 4.68	130.36 ± 6.79	134.44 ± 4.36	0.68 NS	0.614
Cost of production / kg egg mass (Rs.)	139.24 ± 2.99	142.92 ± 3.67	133.97 ± 5.02	140.35 ± 7.59	144.52 ± 4.75	0.64 NS	0.636
Feed cost /dozen egg (Rs)	18.35 ± 0.38	18.56 ± 0.29	17.84 ± 0.74	18.57 ± 0.85	18.93 ± 0.59	0.44 NS	0.779
Cost of production / dozen egg (Rs.)	19.72 ± 0.41	19.92 ± 0.33	19.17 ± 0.80	20.00 ± 0.96	20.35 ± 0.64	0.425 NS	0.789
Cost of production per egg (Rs)	1.644 ± 0.03	1.660 ± 0.03	1.597 ± 0.07	1.667 ± 0.08	1.696 ± 0.05	0.425 NS	0.789
Benefit- cost ratio	1.455 ± 0.02	1.449 ± 0.04	1.538 ± 0.07	1.449 ± 0.07	1.406 ± 0.05	0.91NS	0.472

Each value is the mean of 12 observations. Values in each row having a common superscript do not differ significantly.

*(P<0.05) ** (P<0.01) NS (P>0.05) S.E – Standard error, mm- millimeter, Rs-Indian Rupee

T1- 100 per cent shell grit, T2- 100 per cent 2 mm stone grit, T3- 100 per cent 4 mm stone grit, T4- 50 per cent 2 mm stone grit + 50 per cent shell grit, T5- 4 mm stone grit + 50 per cent shell grit

CONCLUSION

Replacing shell grit with stone grit of varying particle sizes and levels did not significantly affect carcass traits (live weight, dressing per cent, organ yields, intestinal measures) or tibia bone characteristics (weight, length, ash content, calcium per cent, phosphorous per cent and breaking strength). Serum calcium, phosphorous, alkaline phosphatase levels were also unaffected. The economic parameters such as feed cost per kg egg mass or dozen egg, cost of production per kg egg mass or dozen egg were not significantly influenced by the source, particle size of calcium in the diet. It can be concluded that the stone grit is efficient to supply adequate calcium for growth of bone and muscles as that of shell grit and hence the shell grit can be completely replaced with stone grit of size 4 mm in the feed of egg type Japanese quails without compromising the bone quality and carcass yield.

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