

## Effect of replacement of limestone powder with stone grit in diet on production performance and mineral retention in meat type Japanese quails

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### ABSTRACT

The effect of replacement of limestone powder (LSP) with stone grit (SG) was studied in Japanese quails for 4 weeks on production performance, cost effectiveness and mineral retention. In this study, 360 days-old chicks were randomly distributed to 5 dietary treatments with 6 replicates having 12 birds each in a completely randomized design. LSP was replaced with 2 mm and 4 mm sized SG at 50% and 100% level in the treatment groups, such that 100% LSP in T<sub>1</sub> (control), 100% SG of 4 mm particle size in T<sub>2</sub>, 100% SG of 2 mm in T<sub>3</sub>, 50% LSP plus 50% 4 mm stone grit in T<sub>4</sub> and 50% LSP plus 50% 2 mm SG in T<sub>5</sub>. Production parameters such as body weight, feed intake, feed efficiency, livability were recorded for four weeks. Metabolic trial was conducted to estimate the calcium, phosphorous and dry matter retention. Birds fed with 100% of either 2 mm or 4 mm stone grit (T<sub>2</sub> and T<sub>3</sub>) resulted in significantly higher body weight and body weight gain as compared with only 50% replacement groups (T<sub>4</sub> and T<sub>5</sub>), but did not significantly differ with the control (T<sub>1</sub>). Cumulative feed intake of birds fed with 4 mm SG (T<sub>2</sub>) was significantly higher than 2 mm fed birds (T<sub>3</sub>). But, feed efficiency, livability, feed cost per kg live weight, cost of production per kg live weight, calcium, phosphorous and dry matter retention per cent were not significantly different among the treatment groups. The study concluded that stone grit can efficiently supply calcium for proper growth of muscles and bones at par with the limestone powder in terms of body weight gain and feed efficiency. So, the limestone powder can be completely replaced by stone grit of 2 mm particle size in the diet of Japanese quails to get optimum production performance.

**Keywords:** Calcium, Japanese quails, Particle size, Production performance, Stone grit

Japanese quail farming has become popular in Tamil Nadu as they provide quick return on investment, low fat meat and high vitamin eggs with the population of 14.18 lakhs in Tamil Nadu as per 20<sup>th</sup> Livestock Census in 2019, whereas in India, it was 48.86 lakhs which shows the interest build up over the Japanese quail farming. The meat of diversified birds gets great momentum at this time to satisfy need of protein source by developing hybrid varieties in the market. To exploit the genetic potential, well balanced nutrients especially the mineral calcium ensures optimal growth, health and productivity.

The bioavailability and *in vitro* solubility of calcium differs with the particle size. Fine size of calcium source with higher solubility would impact the bioavailability of both calcium and phosphorus by the formation of calcium-phytate complexes which could lessen the action of phytase (Tamim *et al.* 2004) and also protein digestion by disrupting the acidic environment for effective pepsin activity. Therefore, the particle size of the calcium source is essential to optimize the bioavailability and retention of these minerals, along with better protein digestibility.

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Feeding grit stones would facilitate gizzard development, improve feed grinding, and lower the gizzard pH. Limestone powder (LSP) is commonly used in the ration as fine calcium source containing about 37% calcium in the form of calcium carbonate. Stone grit (SG) available in various particle sizes at lower cost in the market can be an alternative source of calcium as the concentrate and form of calcium is same as that of calcite. However, studies on feeding of coarse particle source of calcium in meat-type Japanese quails are lacking and created a knowledge gap in their effectiveness to improve their growth and mineral retention.

The objective of this research was to study the effect of replacing the fine limestone powder with coarse stone grit of different particle sizes (2 mm and 4 mm) at 50% and 100% levels in meat type Japanese quail ration on production performance, cost effectiveness and mineral retention.

### MATERIALS AND METHODS

The biological experiment was conducted at Poultry farm complex, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. In this experiment, 360 numbers of day-old unsexed “TANUVAS Namakkal Quail-1” chicks were reared in a completely randomized

design containing five treatment groups with six replicates of 12 birds in each. This study was approved by the Institutional Animal Ethics Committee (IEAC) of Veterinary College and Research Institute, Namakkal (No.16/VCRI/NKL/2024).

The dietary calcium of 1% was provided to dietary treatments as follows.  $T_1$  -100% LSP (control),  $T_2$  -100% SG with 2 mm size for first 2 weeks, then 4 mm size for next 2 weeks,  $T_3$  -100% SG with 2 mm size for 4 weeks,  $T_4$  -50% LSP plus 50 % SG with 2 mm size for first 2 weeks then 4 mm size for next 2 weeks,  $T_5$  -50% LSP plus 50 % SG with 2 mm for 4 weeks.

The birds were reared under standard uniform management conditions in cage system up to four weeks period. The iso-caloric and iso-nitrogenous basal experimental diet was formulated for both starter (0 to 2 weeks) and finisher phase (2 to 4 weeks). The fine calcium source in the diet, calcite was replaced with stone grit of particle sizes, 2 mm and 4 mm at two different levels, 50% and 100%. All birds were fed *ad-libitum* on a maize-soya based diet containing 1% calcium and 0.40% non-phytate phosphorus, as per Japanese quail breeder specifications (Thomas *et al.* 2022).

Feed intake and mortality were recorded daily. Body weight and residual feed were recorded at weekly intervals. Production performances like body weight and body weight gain, feed conversion ratio, livability were calculated for each replicate. Livability percentage was calculated every week on cumulative basis by the following method:

$$\text{Livability \%} = \frac{\text{No. of birds alive at the end of each week}}{\text{No. of birds initially housed}} \times 100$$

The cost effectiveness was assessed by the parameters such as feed cost per kg live weight, cost of production per kg live weight, cost of production per bird and benefit-cost ratio. Feed cost per kg live weight was calculated by multiplying actual feed cost per kg and feed efficiency at end of fourth week. Cost of production per bird was calculated by adding day old chick cost, feed cost, brooding cost and other inputs. Cost of production per kg live weight was calculated by adding feed cost and chick cost per kg live weight. Chick cost per kg live weight was calculated by dividing the total chick cost by total weight of birds (kg) at the end of fourth week in each replicate. Benefit : cost ratio was calculated by dividing the total income by total expenses in each replicate. The cost of stone grit and limestone powder per kg were Rs 4.30 and Rs 5.18 respectively. The chick cost was Rs 7 per chick. The brooding and maintenance cost per bird was assumed as Rs 2 per bird. The sale price of live bird was Rs 35 per bird or Rs 200/kg live weight.

At the end of 4 weeks, a metabolic trial was conducted with 270 birds which were divided into five treatments with 6 replicates having 9 birds (5 males and 4 females) in each. The quantified treatment specific experimental feed was given to all treatments at the same time. Dry polythene sheet was spread under the cages with clear cut demarcations for

collection of faeces at 24 hours interval for 3 days. Daily feed and cumulative feed intake were measured. The intake of calcium and phosphorus was calculated by multiplying the feed intake with its Ca and P content, divided by 100 (Pastore *et al.* 2012). The fresh weight of excreta was taken in each replicate. An aliquot of 100 g was prepared from each sample and dried in hot air oven at 55°C for 72 hours. After exposed to air to attain equilibrium, aliquots were weighed and ground. Subsequently, the dry matter, and Ca and P were determined as per the method of AOAC (2019).

The data collected on various parameters were statistically analyzed by one-way ANOVA method in SPSS 20<sup>th</sup> version and the means of different experimental groups were tested for statistical significance by Duncan's multiple range test (Duncan 1955).

## RESULTS AND DISCUSSION

**Body weight and body weight gain:** The Japanese quails fed with 100% replacement of either 2 mm or 4 mm size SG ( $T_2$  and  $T_3$ ) resulted in significantly ( $p \leq 0.01$ ) higher body weight and body weight gain at 2, 3, and 4 weeks of age as compared to Japanese quails that received 50% replacement ( $T_4$  and  $T_5$ ), but it was not significant with control ( $T_1$ ) indicating that complete replacing of fine LSP with coarse SG of both 2 mm and 4 mm size did not affect the growth performance in terms of body weight body as long as the optimum calcium level in the diet was maintained (Table 1). Similar non significant results were observed in layers (Olgun *et al.* 2015), in broilers (Rezvani *et al.* 2019, Poudel *et al.* 2022) after feeding with different combinations of finer and coarser limestone and in European quails (Mendonca *et al.* 2022) by feeding five different calcium sources.

The Japanese quails fed with stone grit replacement at 50% ( $T_4$  and  $T_5$ ) had significantly ( $p \leq 0.01$ ) lower body weight and body weight gain as compared with 100% replacement groups or control suggesting that partial replacement (50%) was less effective due to failure of adaptation and poor appetite. However, Karim and Abdulla (2024) found that replacement of limestone with coarser eggshell at 0, 50 and 100% levels did not significantly affect body weight or weight gain in broilers which is contrast to the present results.

The present study revealed that the particle size of stone grit did not influence the body weight in both replacement levels. In contrast, significantly higher body weight was observed in European quails (Leao *et al.* 2020) and in layers (Khanal *et al.* 2020) upon feeding coarser limestone. But, Manangi and Coon (2007) demonstrated that broiler chicks fed medium  $\text{CaCO}_3$  particle sizes (137-388  $\mu\text{m}$ ) had significantly increased weight gains when compared to smaller (28  $\mu\text{m}$ ) or larger (1306  $\mu\text{m}$ ) particles. The beneficial effects of coarser calcium source were explained by better protein digestion (Selle *et al.* 2009), better calcium and phosphorous availability (Tamim *et al.* 2004), better fat absorption (Hamdi *et al.* 2018) and better grinding and enzymatic action in the gizzard (Rutkowski and Wiaz 2001).

Table.1 Mean ( $\pm$ S.E.) of body weight (g), body weight gain (g) and feed conversion ratio of meat type Japanese quails fed diet with different sizes and inclusion levels of stone grit

| Age (in weeks)                        | T <sub>1</sub>                  | T <sub>2</sub>                 | T <sub>3</sub>                   | T <sub>4</sub>                 | T <sub>5</sub>                  | p-Value     |
|---------------------------------------|---------------------------------|--------------------------------|----------------------------------|--------------------------------|---------------------------------|-------------|
| <i>Body weight (g)</i>                |                                 |                                |                                  |                                |                                 |             |
| Day 1                                 | 9.21 $\pm$ 0.03                 | 9.16 $\pm$ 0.03                | 9.18 $\pm$ 0.06                  | 9.21 $\pm$ 0.05                | 9.18 $\pm$ 0.05                 | 0.935       |
| I                                     | 24.44 <sup>C</sup> $\pm$ 1.24   | 25.03 <sup>C</sup> $\pm$ 0.45  | 23.99 <sup>BC</sup> $\pm$ 1.02   | 20.72 <sup>A</sup> $\pm$ 0.59  | 21.77 <sup>AB</sup> $\pm$ 0.67  | 0.005       |
| II                                    | 56.26 <sup>B</sup> $\pm$ 2.03   | 59.70 <sup>B</sup> $\pm$ 0.72  | 57.10 <sup>B</sup> $\pm$ 1.58    | 48.38 <sup>A</sup> $\pm$ 1.62  | 49.13 <sup>A</sup> $\pm$ 0.60   | $\leq$ 0.01 |
| III                                   | 103.08 <sup>B</sup> $\pm$ 2.43  | 103.78 <sup>B</sup> $\pm$ 2.23 | 105.29 <sup>B</sup> $\pm$ 2.77   | 94.23 <sup>A</sup> $\pm$ 1.22  | 95.51 <sup>A</sup> $\pm$ 2.65   | 0.005       |
| IV                                    | 170.54 <sup>B</sup> $\pm$ 2.48  | 174.74 <sup>B</sup> $\pm$ 1.34 | 171.38 <sup>B</sup> $\pm$ 3.38   | 161.95 <sup>A</sup> $\pm$ 2.44 | 162.95 <sup>A</sup> $\pm$ 1.98  | 0.003       |
| <i>Body weight gain (g)</i>           |                                 |                                |                                  |                                |                                 |             |
| I                                     | 15.23 <sup>C</sup> $\pm$ 1.24   | 15.86 <sup>C</sup> $\pm$ 0.43  | 14.81 <sup>BC</sup> $\pm$ 0.98   | 11.52 <sup>A</sup> $\pm$ 0.62  | 12.59 <sup>AB</sup> $\pm$ 0.64  | 0.004       |
| II                                    | 47.05 <sup>B</sup> $\pm$ 2.02   | 50.53 <sup>B</sup> $\pm$ 0.70  | 47.92 <sup>B</sup> $\pm$ 1.54    | 39.18 <sup>A</sup> $\pm$ 1.64  | 39.95 <sup>A</sup> $\pm$ 1.59   | $\leq$ 0.01 |
| III                                   | 93.87 <sup>B</sup> $\pm$ 2.43   | 94.62 <sup>B</sup> $\pm$ 2.23  | 96.11 <sup>B</sup> $\pm$ 2.74    | 85.02 <sup>A</sup> $\pm$ 1.23  | 86.34 <sup>A</sup> $\pm$ 2.62   | 0.005       |
| IV                                    | 161.33 <sup>B</sup> $\pm$ 2.48  | 165.58 <sup>B</sup> $\pm$ 1.33 | 162.19 <sup>B</sup> $\pm$ 3.34   | 152.74 <sup>A</sup> $\pm$ 2.44 | 153.78 <sup>A</sup> $\pm$ 1.98  | 0.003       |
| <i>Cumulative feed consumption(g)</i> |                                 |                                |                                  |                                |                                 |             |
| I                                     | 43.18 <sup>bc</sup> $\pm$ 1.35  | 45.58 <sup>c</sup> $\pm$ 1.57  | 41.15 <sup>abc</sup> $\pm$ 2.21  | 38.18 <sup>a</sup> $\pm$ 1.53  | 39.60 <sup>ab</sup> $\pm$ 0.69  | 0.02        |
| II                                    | 137.76 <sup>bc</sup> $\pm$ 3.77 | 144.03 <sup>c</sup> $\pm$ 1.87 | 134.58 <sup>abc</sup> $\pm$ 4.46 | 126.52 <sup>a</sup> $\pm$ 2.76 | 130.68 <sup>ab</sup> $\pm$ 3.49 | 0.013       |
| III                                   | 282.29 <sup>ab</sup> $\pm$ 5.99 | 287.68 <sup>b</sup> $\pm$ 3.21 | 267.51 <sup>a</sup> $\pm$ 6.36   | 267.03 <sup>a</sup> $\pm$ 5.69 | 267.52 <sup>a</sup> $\pm$ 4.97  | 0.024       |
| IV                                    | 460.93 <sup>ab</sup> $\pm$ 7.13 | 472.06 <sup>b</sup> $\pm$ 2.92 | 449.37 <sup>a</sup> $\pm$ 8.33   | 446.12 <sup>a</sup> $\pm$ 6.38 | 444.37 <sup>a</sup> $\pm$ 5.78  | 0.024       |
| <i>Feed conversion ratio</i>          |                                 |                                |                                  |                                |                                 |             |
| I                                     | 2.92 <sup>bc</sup> $\pm$ 0.52   | 2.88 <sup>c</sup> $\pm$ 0.30   | 2.80 <sup>abc</sup> $\pm$ 0.21   | 3.37 <sup>a</sup> $\pm$ 0.63   | 3.18 <sup>ab</sup> $\pm$ 0.37   | 0.156       |
| II                                    | 2.95 <sup>AB</sup> $\pm$ 0.13   | 2.85 <sup>A</sup> $\pm$ 0.06   | 2.81 <sup>A</sup> $\pm$ 0.05     | 3.25 <sup>BC</sup> $\pm$ 0.11  | 3.29 <sup>C</sup> $\pm$ 0.14    | 0.005       |
| III                                   | 3.01 <sup>b</sup> $\pm$ 0.07    | 3.05 <sup>b</sup> $\pm$ 0.08   | 2.79 <sup>a</sup> $\pm$ 0.04     | 3.14 <sup>b</sup> $\pm$ 0.05   | 3.11 <sup>b</sup> $\pm$ 0.09    | 0.014       |
| IV                                    | 2.86 $\pm$ 0.05                 | 2.85 $\pm$ 0.02                | 2.77 $\pm$ 0.02                  | 2.92 $\pm$ 0.05                | 2.89 $\pm$ 0.04                 | 0.094       |

Each value is the mean of 6 observations. Values in each row having common superscript do not differ significantly; Superscripts of lower-case letters denote the significance at  $p \leq 0.05$  and uppercase letters for  $p \leq 0.01$

But these beneficial effects were not enough to result in significantly higher body weight or body weight gain in this study of Japanese quails suggesting that the established calcium and phosphorous homeostasis mechanisms had balanced the release of calcium ions as well as gizzard pH differences. This study concluded that 100% replacement of LSP with 2 mm and 4 mm SG was better in growth performance but, not surpassed the control.

**Feed intake:** There was no significant difference ( $p \geq 0.05$ ) in feed intake between T<sub>2</sub> and T<sub>3</sub> in the first and second week of age, whereas significantly ( $p \leq 0.05$ ) increased feed intake (7.54% and 5.05%) was found in T<sub>2</sub> during third and fourth weeks of age as compared with T<sub>3</sub>. This clearly indicated that the particle size of the stone grit significantly influenced the feed intake since the Japanese quails in T<sub>2</sub> were given 4 mm stone grit in third and fourth weeks. The increased feed intake might be due to substantial impact of particle size on the birds' appetite for calcium requirements. Similar results were found by deAraujo *et al.* (2011), Saki *et al.* (2019) and Poudel *et al.* (2022) who reported that increased feed intake in layers due to larger particle size as compared to finer particle size, whereas higher feed intake with fine particle was observed by Geraldo *et al.* (2006) which was contradictory to the present findings. However, no significant difference in feed intake was observed in Japanese quails (Berto *et al.* 2007), European quails (Leao *et al.* 2020, Mendonca *et al.* 2022)

and in layers (Pizzolante *et al.* 2009, Pacheco *et al.* 2020) with respect to particle size of calcium source. In this study also, there was no significant difference between control (T<sub>1</sub>) and 100% replacement groups (T<sub>2</sub> and T<sub>3</sub>) which inferred that Japanese quails can adapt themselves by changing the calcium homeostasis mechanisms to balance the changes in the particle size of coarser stone grit as that of finer calcite without significant change in feed intake.

The cumulative feed intake of 50% replacement groups T<sub>4</sub> and T<sub>5</sub> had significantly lower feed intake ( $P \leq 0.05$ ) as compared with control (T<sub>1</sub>) and 100% replacement group (T<sub>2</sub>). This might be due to the difference in the bird's appetite for calcium when both fine and coarse calcium sources were fed. This result coincided with the findings of Maranan *et al.* (2021) that 75% replacement of limestone with eggshell significantly reduced the feed intake in broilers. However, no significant difference in feed intake was recorded by replacing the finer with coarser limestone up to 70% (Pelicia *et al.* 2007), finer limestone with marine calcium source up to 60% in layers (Pelicia *et al.* 2009) and limestone with eggshell up to 100% in broilers (Karim and Abdulla 2024). This study suggested that the SG can be used as an alternative to the LSP as 100% replacement rather than 50 % replacement for optimum feed intake of Japanese quails.

**Feed conversion ratio:** The replacement of LSP with 2 mm and 4 mm size SG at 100% and 50% levels had no

significant effect on feed conversion ratio at 4 weeks of age ( $p \geq 0.05$ ). Similar non-significant observations on feed conversion ratio were noticed by Sousa *et al.* (2019) in layers, Mendonca *et al.* (2022) in non-sexed European quails and Karim and Abdulla (2024) in broilers. However, at 3 weeks of age, 100% (2 mm) SG replacement group ( $T_3$ ) had significantly better feed conversion ratio ( $p \leq 0.01$ ) compared to other replacement groups and control. The improved feed efficiency on feeding coarse calcium source might be due to better digestibility of protein (Walk *et al.* 2012) by maintaining ideal pH in the proventriculus for pepsin activity and more bioavailability of phosphorous during the younger age, as the younger birds lack endogenous phytase enzyme to breakdown calcium phytate complexes (Tamim *et al.* 2004). Similar results of significantly better feed efficiency by feeding coarse calcium sources were demonstrated by Barshan *et al.* (2019) and Hu *et al.* (2020) in broilers, Wang *et al.* (2014) in ducks and Leao *et al.* (2020) in European quails indicating that calcium source and particle size influenced the feed efficiency across the species. However, the numerically better feed efficiency found in 100% stone grit replacement group ( $T_3$ ) was not significant ( $p \geq 0.05$ ) when compared with both 50% and control group at 4 weeks of Japanese quails.

**Livability:** The livability % was not significantly influenced by the replacement level or particle size ( $P > 0.05$ ) in this study (Table 2). The result was consistent with previous findings of Pelicia *et al.* (2007) in Hy-Line layers fed with diets replacing of calcitic limestone (0.18mm) with marine calcium source (0.4 mm) at 0, 15, 30, 45, and 60% level. Pizzolante *et al.* (2009) in layers feeding with different particle sizes of limestone during second production cycle and Fallah *et al.* (2018) in broilers by feeding limestone and oyster shell as calcium source. On the contrary, Manangi and Coon (2007) found the highest mortality percentage in broilers fed with the largest particle-sized limestone (1306  $\mu$ m), whereas Kermanshahi and Golian (2011) reported that the mortality of laying hens in limestone treatment was greatest (13.89%) and lowest in oyster shell + filter mud treatment. The mortality

observed in this study was due to some other factors like dehydration, heat stress, cannibalism etc. So, the particle size of calcium source and replacement levels had no adverse effects leading to mortality.

**Cost effectiveness:** The feed cost per kg live weight did not differ significantly ( $p \geq 0.05$ ) by feeding SG at different replacement levels and particle sizes. But, the cost of production per kg live weight and benefit : cost ratio were significantly better ( $p \leq 0.05$ ) in 100% SG replacement ( $T_3$ ) group when compared to 50 % replacement groups. The reason might be due to better feed efficiency and livability in  $T_3$ . The production cost per bird was significantly reduced (3.8, 4.1%) in 50% replacement groups  $T_4$  and  $T_5$  than 100% replacement group  $T_2$  and not lower than control group. The reason might be due to reduced cumulative feed consumption in  $T_4$  and  $T_5$ . Another finding was that the replacement of SG at either 50% or 100% did not reduce the cost of production, because the cost of LSP and SG per kg was more or less same (Rs. 5.18 Vs 4.30).

The study of Moura *et al.* (2020) in Japanese quail layers was in parallel with the present findings in that partial substitution (25% and 50%), replacing limestone with eggshell powder improved ( $p \leq 0.05$ ) the economic feed efficiency index. In contrast, Islam and Nishibori (2021) concluded that 8% eggshell group performed the best among the different dietary groups in terms of production cost and net profit in layers. Muthukumar *et al.* (2017) found that the cost of production per bird in small, medium and large Japanese quail farms were Rs 28.16, 27.88 and 25.21 respectively, whereas the average production cost per bird in control, 100% and 50% replacement groups were Rs 27.00, 26.98 and 26.39. The higher cost of production per bird as compared with the present study might be due to the addition of interest amount on the fixed cost and depreciation cost. The replacement of LSP with SG at 100% and 50% level in the diet of Japanese quails did not significantly ( $p \geq 0.05$ ) improve the economical parameters when compared to control group because the feed efficiency and feed cost per kg live weight were insignificant in all treatment groups at the marketing age of 4 weeks.

Table 2. Mean ( $\pm$ S.E.) livability (%) and cost effectiveness parameters of meat type Japanese quails fed diet with different sizes and inclusion levels of stone grit

| Age<br>(in weeks)                        | $T_1$                            | $T_2$                           | $T_3$                          | $T_4$                          | $T_5$                           | $p$ -Value |
|------------------------------------------|----------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|------------|
| <i>Livability %</i>                      |                                  |                                 |                                |                                |                                 |            |
| I                                        | 100                              | 100                             | 100                            | 100                            | 100                             | -          |
| II                                       | 100                              | 98.61 $\pm$ 1.39                | 100                            | 98.61 $\pm$ 1.39               | 100                             | 0.567      |
| III                                      | 97.22 $\pm$ 1.76                 | 98.61 $\pm$ 1.39                | 100                            | 97.22 $\pm$ 1.76               | 100                             | 0.339      |
| IV                                       | 97.22 $\pm$ 1.76                 | 98.61 $\pm$ 1.39                | 100                            | 97.22 $\pm$ 1.76               | 98.21                           | 0.673      |
| <i>Cost Effectiveness</i>                |                                  |                                 |                                |                                |                                 |            |
| Feed cost per kg live weight (Rs.)       | 111.65 $\pm$ 1.91                | 111.30 $\pm$ 0.78               | 108.18 $\pm$ 0.79              | 114.12 $\pm$ 1.82              | 112.87 $\pm$ 1.67               | 0.091      |
| Production cost per kg live weight (Rs.) | 154.02 <sup>abc</sup> $\pm$ 2.98 | 151.98 <sup>ab</sup> $\pm$ 1.02 | 149.10 <sup>a</sup> $\pm$ 1.36 | 158.71 <sup>c</sup> $\pm$ 2.43 | 156.51 <sup>bc</sup> $\pm$ 2.02 | 0.029      |
| Production Cost per bird (Rs.)           | 27.00 <sup>ab</sup> $\pm$ 0.28   | 27.43 <sup>b</sup> $\pm$ 0.11   | 26.54 <sup>a</sup> $\pm$ 0.33  | 26.42 <sup>a</sup> $\pm$ 0.25  | 26.35 <sup>a</sup> $\pm$ 0.23   | 0.025      |
| Benefit : cost ratio                     | 1.26 <sup>abc</sup> $\pm$ 0.02   | 1.27 <sup>bc</sup> $\pm$ 0.01   | 1.29 <sup>c</sup> $\pm$ 0.01   | 1.23 <sup>a</sup> $\pm$ 0.02   | 1.24 <sup>ab</sup> $\pm$ 0.01   | 0.026      |

Each value is the mean of 6 observations; Value in each row having common superscript do not differ significantly

**Mineral retention:** The particle size and replacement levels of stone grit had no significant ( $p \geq 0.05$ ) difference on calcium, phosphorous and dry matter retention percentage in Japanese quails which was similar to the findings of Manangi and Coon (2007) and Khalil and Anwar (2009) who noticed that the particle size of limestone or oyster shell had no significant effect on calcium and phosphorous retention in broilers. On the contrary, significant improvement ( $P \leq 0.05$ ) in phosphorous retention in layers was found by Lichovnikova (2007) with ratio of 32:68 fine limestone: eggshell. Significantly higher calcium retention ( $P < 0.01$ ) was observed in broilers by Rezvani *et al.* (2019) on feeding eggshell and by Hakami *et al.* (2022) on feeding the marine calcium source Celtical at 0.2% and 0.4% and in layers by Roland and Bryant (2000) by feeding oyster shell.

However, significantly ( $p \leq 0.01$ ) higher dry matter, calcium and phosphorous intake and retention (g/day) were found in 100% replacement group  $T_2$  when compared to 50% replacement group  $T_4$ . This higher intake might be due to greater body weight at 4 weeks of age. The calcium retention % was numerically higher in 100% replacement groups  $T_2$  and  $T_3$  when compared to other treatment groups regardless of the particle size of stone grit indicating that particle size within this range (2 mm to 4 mm) did not strongly influence calcium retention. This result differed from the previous findings that feeding finer particles of limestone had more retention of calcium and phosphorous in broilers with fine particle sizes (Majeed *et al.* 2020, Hu *et al.* 2022).

The phosphorous retention (g/day) was significantly higher ( $p \leq 0.05$ ) in  $T_2$  when compared with control indicating that the coarser particles of calcium source enhanced calcium and phosphorous availability as evidenced by Kim *et al.* (2018) and Manangi and Coon (2007) due to

lesser formation of calcium phosphate (Selle *et al.* 2009) and calcium-phytate complex (Tamim *et al.* 2004) and this concept was supported by the findings of Manangi *et al.* (2018) who noticed that feeding larger particle-size reduced the phosphorus content of broiler excreta without affecting calcium content.

The excretion of calcium, phosphorous and dry matter were not significant ( $p \geq 0.05$ ) in all treatments indicating that replacement with stone grit irrespective of particle size promoted the absorption of dry matter and minerals as equivalent to that of control group (Table 3). This finding was in contrast with Moura *et al.* (2020) who reported that replacing limestone with eggshell powder in Japanese quail diet increased calcium excretion linearly with higher calcium wastage at 100% replacement. Rezvani *et al.* (2019) found that dry matter digestibility was significantly higher in broilers replaced with eggshell as calcium source compared to birds with oyster shells ( $p \leq 0.05$ ). This was contradictory with the present study since the dry matter retention was not significant within the stone grit replaced groups and the control group.

There seems to be a mixed consensus on particle size effects, with some studies showing that finer particles improved calcium and phosphorus digestibility and some authors proved coarser particles improved the calcium and phosphorous retention. The differences might be due to the variation in the *in-vivo* solubility, pH of the gizzard, calcium and phosphorous levels in the feed, age, species and calcium requirements of the body. This study inferred that particle size did not significantly ( $p \geq 0.05$ ) impact calcium, phosphorus retention or excretion as long as the calcium and non-phytate phosphorous in the feed was maintained at 1% and 0.4%, respectively.

The study concluded that Japanese quails fed, a diet in which limestone powder was fully (100%) replaced with

Table 3. Mean ( $\pm$ S.E.) of dry matter and mineral retention studies of meat type Japanese quails fed diet with different sizes and inclusion levels of stone grit

| Mineral retention            | $T_1$                           | $T_2$                          | $T_3$                           | $T_4$                          | $T_5$                           | p-Value     |
|------------------------------|---------------------------------|--------------------------------|---------------------------------|--------------------------------|---------------------------------|-------------|
| <i>Calcium</i>               |                                 |                                |                                 |                                |                                 |             |
| Calcium intake (g/day)       | 3.032 <sup>AB</sup> $\pm$ 0.044 | 3.335 <sup>c</sup> $\pm$ 0.068 | 3.255 <sup>BC</sup> $\pm$ 0.061 | 2.877 <sup>A</sup> $\pm$ 0.126 | 3.240 <sup>BC</sup> $\pm$ 0.038 | 0.001       |
| Calcium excreted g/day)      | 1.86 $\pm$ 0.125                | 1.892 $\pm$ 0.021              | 1.813 $\pm$ 0.059               | 1.975 $\pm$ 0.098              | 1.977 $\pm$ 0.115               | 0.673       |
| Calcium retention (g/day)    | 1.165 <sup>ab</sup> $\pm$ 0.118 | 1.443 <sup>b</sup> $\pm$ 0.069 | 1.442 <sup>b</sup> $\pm$ 0.036  | 0.902 <sup>a</sup> $\pm$ 0.194 | 1.263 <sup>ab</sup> $\pm$ 0.117 | 0.02        |
| Calcium retention (%)        | 38.46 $\pm$ 3.98                | 43.16 $\pm$ 1.32               | 44.33 $\pm$ 1.12                | 30.46 $\pm$ 5.09               | 38.97 $\pm$ 3.46                | 0.059       |
| <i>Phosphorus</i>            |                                 |                                |                                 |                                |                                 |             |
| Phosphorus intake (g/day)    | 1.408 <sup>A</sup> $\pm$ 0.020  | 1.623 <sup>B</sup> $\pm$ 0.035 | 1.602 <sup>B</sup> $\pm$ 0.021  | 1.398 <sup>A</sup> $\pm$ 0.061 | 1.577 <sup>B</sup> $\pm$ 0.019  | $\leq 0.01$ |
| Phosphorus excreted (g/day)  | 0.888 $\pm$ 0.052               | 0.908 $\pm$ 0.040              | 1.012 $\pm$ 0.054               | 0.942 $\pm$ 0.037              | 0.975 $\pm$ 0.051               | 0.377       |
| Phosphorus retention (g/day) | 0.520 <sup>a</sup> $\pm$ 0.043  | 0.715 <sup>b</sup> $\pm$ 0.020 | 0.590 <sup>ab</sup> $\pm$ 0.041 | 0.457 <sup>a</sup> $\pm$ 0.078 | 0.602 <sup>ab</sup> $\pm$ 0.057 | 0.021       |
| Phosphorus retention (%)     | 37.01 $\pm$ 3.22                | 44.16 $\pm$ 1.64               | 36.95 $\pm$ 2.80                | 32.00 $\pm$ 4.00               | 38.09 $\pm$ 3.37                | 0.133       |
| <i>Dry matter</i>            |                                 |                                |                                 |                                |                                 |             |
| Dry matter intake (g/day)    | 192.55 <sup>B</sup> $\pm$ 2.78  | 196.88 <sup>B</sup> $\pm$ 4.05 | 191.94 <sup>B</sup> $\pm$ 3.56  | 169.83 <sup>A</sup> $\pm$ 7.43 | 191.33 <sup>B</sup> $\pm$ 2.25  | 0.002       |
| Dry matter excreted (g/day)  | 64.48 $\pm$ 3.31                | 64.15 $\pm$ 1.26               | 64.39 $\pm$ 1.99                | 64.91 $\pm$ 2.49               | 66.47 $\pm$ 3.97                | 0.977       |
| Dry matter retention (g/day) | 128.07 <sup>B</sup> $\pm$ 2.72  | 132.73 <sup>B</sup> $\pm$ 3.34 | 127.55 <sup>B</sup> $\pm$ 2.76  | 104.92 <sup>A</sup> $\pm$ 9.00 | 124.86 <sup>B</sup> $\pm$ 5.18  | 0.008       |
| Dry matter retention (%)     | 66.55 $\pm$ 1.49                | 67.39 $\pm$ 0.52               | 66.45 $\pm$ 0.80                | 61.31 $\pm$ 2.46               | 65.20 $\pm$ 2.21                | 0.119       |

Each value is the mean of 6 observations, Values in each row having common superscript do not differ significant. Superscripts of lower case letters denote the significance at  $p \leq 0.05$  and uppercase letters for  $p \leq 0.01$

stone grit, performed better than those receiving only a 50% replacement. As the feed efficiency, livability, cost of production, and benefit : cost ratio of birds fed with 2 mm stone grit were numerically superior to those fed with 4 mm grid, it is recommended that limestone powder can be safely replaced with 2 mm stone grit at a 100% inclusion level in the diet of meat type Japanese quails to achieve optimum production performance.

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