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# Proximate and mineral composition of Japanese quail egg and its possible role in bone healing

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Quail eggs are known to be highly nutritious. In the first study, the nutritional and mineral composition of quail eggs was evaluated by determining the proportion of moisture, total ash, lipids, nitrogen, crude protein and carbohydrate in the egg. The second phase was to determine the role of quail eggs in bone healing. Freshly laid Japanese quail eggs were purchased from a research institute in Plateau state, Nigeria. Five eggs were randomly selected and used for the evaluation of the nutritional and mineral composition of eggs. They were subjected to different methods to obtain the proximate and nutritive content. The moisture content in the egg was 65.5 %, total ash was 8.5 %, lipid was 1.5 %, nitrogen was 0.75 %, crude protein was 4.72 %, and carbohydrate was 19.78 %. For the mineral content, sodium was 42.5 mg, potassium was 80 mg, phosphorus was 6.49 mg, calcium was 0.9 mg, and magnesium was 1.3 mg. In the second study, 12 male New Zealand white rabbits with an average age of 7–8 months were used. They were separated into 2 groups comprising 6 rabbits per group. Rabbits in group A had a cylindrical trephine drill to create a 3.5 mm diameter defect on the lateral distal epicondyle of the left femur and were monitored for 12 weeks. Faster healing was observed in the group administered quail egg. At week 10, the radiographic score of the quail egg treated group [4(3-4)] was significantly ($P < 0.05$) higher compared to the control group with 2(2-3). At week 12, complete healing was observed in the quail egg group [4(4-4)]; this was different from the control group that had 2 rabbits yet to be healed. The study shows that Japanese quail egg is nutritious and rich in essential nutrients including calcium and phosphorus and as such it can serve as a nutritional supplement to enhance bone healing.

*Keywords*: Bone healing, Mineral content, Proximal analysis, Quail egg, Supplement

## Introduction

Good nutrition has been known to play a vital role in the body's growth and development. Quail eggs have been reported to have high nutritional content, which makes it valuable in many therapeutic diets for adults (Herranz *et al.,* 2007). Quail egg is considered to be one of the best-known natural treatment products. Chinese medical practitioners have been using quail egg as a treatment for hundreds of years with brilliant

results. More and more people are beginning to show interest in its use as an active natural medicine instead of conventional medicines with so many side effects. It has been reported that people with macronutrient deficiencies should use egg to supplement their diets (Sarkingobir *et al.,* 2020).

According to nutritionists, Quail egg as food is one of the richest in good and essential ingredients, and we all should have at least one a day. It is also known to be the best source of proteins after milk (Vaclavik & Christain, 2008). The Japanese quail (*Coturnix japonica*) is about 20 cm from its beak to its tail. It is also a popular source of meat and eggs in various parts of the world including Nigeria. Its eggs are highly consumed all over the world especially in Asian countries (Omoniyi & Abba 2018). Quail egg has a small weight ranging from 9-12g which is one-fifth of the size of a chicken egg (Altuntas & Sekeroglu, 2008). Despite their small size, they have high nutritional values of about three to four times greater than other eggs and they are rich sources of antioxidants, minerals, and vitamins which provide the body with a lot of nutrition than do other foods (Oluwafemi & Udeh, 2016). They lay up to 350 eggs annually and their eggs are characterized by a variety of shell color patterns which ranges from dark brown to speckled white. Regular consumption of quail eggs has been reported to help fight against many diseases and strengthen the immune system (Herranz *et al.,* 2007). Potassium (K) is needed for the activation of several enzymes. Low intake of it is associated with a high risk of gastrointestinal disorders, arthritis, stroke, infertility, and cancer (Tunsaringkarn *et al.,* 2013). Sodium (Na) and potassium (K) have been documented to be involved in the regulation of osmotic pressure and transmission of nerve impulses (Dieter, 2008). The technique of emission flame photometry is a traditional and simple method for determining sodium and potassium (Bello & Abdu, 2011). Soluble calcium (Ca) compounds are the strength in the skeleton and are essential for bone growth and development. They are essential in blood clotting, muscle contraction, building of the myelin covering of the brain nerve cells which allows faster transmission of electrical signal in the brain and bone and tooth formation (Sarkingobir *et al.,* 2020). Magnesium (Mg) acts as a cofactor of many enzymes (Dieter, 2008). Phosphate (P) acts as a buffer in the plasma and is also used to detect pH levels in the body. It is also important in the regulation and control of enzymes and serves in phospholipids, DNA, and RNA. Side effects of excess intake of P can cause malabsorption of calcium, and skeletal porosity (Skalnaya & Skalny, 2018). Since the information on the nutritional and mineral composition of quail eggs as it relates to health is not adequate to the best of our knowledge, this study aimed to determine the nutritional and mineral contents of quail eggs as a supplement during bone healing, knowing the mineral and nutritive content of quail eggs may solve some health problems even in the area of medicine and bone healing. Quail egg is a universal natural food supplement with no health implications and safe to use. Their high nutrient content, low caloric value and easy digestion make them important in many therapeutic (Herranz *et al.,* 2007). The importance of nutrition in bone health cannot be overstated. Calcium and phosphate supplement appears to be preferable to other bone supplements such as carbonate or citrate Ca salt (Bonjour, 2011). To the best of our knowledge, few works have been documented on quail eggs and healing, such as: nutrient benefits of quail (Tunsaringkarn *et al*., 2013), effects of quail eggs on surgical excisional wound in rabbits has been reported by Oviawe *et al.* (2020) and there is need to test its effect on bones. The present study was aimed at utilizing the nutritional content of Japanese quail eggs in improving bone healing of a defect in a rabbit model.

**Materials and Methods**

*Ethical approval*

Ethical clearance was sought and obtained in accordance with the statutory regulations guiding animal care and use as approved by Ahmadu Bello University Committee on animal Use and Care (ABUCAUC). An approval number (ABUCAUC/2021/096) was issued.

*Experiment one*

Freshly laid eggs (Plate I) used for this study were purchased from National Veterinary Research Institute (NVRI), Vom in Jos and transported to Sokoto during the rainy season in August, 2001. They were identified and subjected to analysis at the Agriculture Chemical Laboratory of Usmanu Danfodiyo University, Sokoto, Nigeria. Proximate analysis was conducted on randomly selected eggs (n=5) to determine the percentages of moisture, total ash, lipid, crude protein and carbohydrate, using the method described by the Association of Officiating Analytical Chemists (AOAC, 2005) as explained below and also according to the method of Bello & Abdu (2011), where he documented that the moisture was measured using the oven dry method, ash was measured using the muffle furnace ashing method,

ether was measured using the soxhlet extraction method using petroleum ether, crude fiber was measured using the muffle ashing method, and crude proteins were measured using the micro-Kjeldahl method.

The moisture content of each whole quail egg sample was evaluated using the AOAC (2005) technique. This technique was done by washing a crucible and drying it at 100°C in an oven to a consistent weight. This was then removed, chilled in a dessicator, and weighed once more. The initial weight was given a name (W1). A measured crucible was filled with around 2 g of the properly mixed egg sample, which was weighed again (W2). The crucible containing the sample was then dried and weighed again after being placed in an oven at 100°C for 45-60 minutes. It was placed back into the oven and weighed again after about 2 hours to confirm that the weight remained consistent (W3).

Then the moisture content was calculated as follows:

$$\% \text{ moisture} = \frac{W2-W1}{W3-W1} \times 100$$

The AOAC (2005) method for determining ash content was used with slight modification. This method involves cleaning and drying a crucible in the oven was used to determine the ash content. After that, it was dried and weighed in a desiccator (W1). After that, a 2 g pulverized whole egg sample was weighed in the crucible (W2). The sample was then placed in a furnace set to 55°C and burned for 8 hours, after which the crucible containing the ash was retrieved and cooled using desiccators before being weighed (W3). The ash content of each egg sample corresponds to the weight of the residue in the crucible.

The total lipid content of a complete quail egg sample was measured using the AOAC (2005) technique with slight modifications. This was done by washing the flasks and adding a tiny amount of anti-bump granules to prevent bumping, 300 $cm^3$ petroleum ether (boiling point 40–60°C) was put into the flask. Soxhlet extraction units were installed with this. The extraction units and the extraction thimbles were weighed. Then it was filled with 2 g of dried quail egg sample and weighed as follows: (W1). The cold-water circulation system was turned on after the thimble was installed in the soxhlet extraction unit. The heating mantle was turned on, and the solvent-fixing refluxing rate was gradually increased and the extraction process took 8 hours. The thimble was removed and dried to a constant weight in a 70°C oven before being weighed as follows: (W2). The percentage by weight of lipid was calculated as

**Plate I**: The quail eggs used in this experiment

$$\text{Lipid (W/W)} = \frac{\text{Weight of lipid extracted}}{\text{Weight of dried sample}} \times 100$$

Determination of nitrogen and crude protein according to AOAC (2005) techniques. This technique involves 3 processes; the digestion, distillation and titration. In the digestion process, the matter was oxidized, and nitrogen was reduced to ammonium sulphate using sulphuric acid. In the distillation process, Sodium hydroxide was used to liberate the ammonia. The ammonia was titrated with hydrochloric acid after being trapped in a (excess) boric acid solution (HCl). In the titration process, the back titration method was used, in which ammonia reacts with boric acid in the receiving flask and the amount of excess acid is measured using HCl titration. The percentage of total nitrogen was calculated, and crude protein was estimated using the standard conversion factor of 6.25. Precisely 2 g of dried quail egg sample was weighed into a 100 $cm^3$ Kjeldah flask, then 1 g of catalyst ($K_2SO4$ and $CuSO_4$) was added to speed up the reaction in the flask at first, while ferritin was added to slow the reaction, and the flask was made more rigorous by occasional rotation of the flask to ensure even digestion while avoiding overheating of the content. After obtaining a clear solution, the sample was transferred to a 100 $cm^3$ volumetric flask and diluted to the mark with distilled water. 10 $cm^3$ of the digested diluted sample was pipetted into Markham semi macro nitrogen after cooling. After that, 10 $cm^3$ of a 40 percent NaOH solution was added. The sample was then distilled to liberate $NH_3$ into a 100 $cm^3$ conical flask containing 10 $cm^3$ of 40 % boric acid and two drops of methyl red indicator. The distillation was continued until the indicator's pink color changed to a greenish color. The control was titrated with 4 percent boric acid, with a shift in color from greenish to pink indicating the end point.

Carbohydrate concentration was also evaluated using the AOAC (2005) technique. The carbohydrate content was calculated by subtracting the moisture content, ash content, total protein, and fat content from 100, which is known as estimation by difference. The mineral makeup of the same 5 Japanese quail eggs was determined at random. Flame photometry (Flame Corning Limited, Halsted Essex England) was used to estimate sodium and potassium levels according to the method described by Sharma & Sarmah (2013). This method is based on the idea that the quail sample drawn into a non-luminous flame will ionize, absorb energy from the flame, and then emit light of a specific wavelength as the excited atoms decay to the ground state. The strength of the emission is proportional to the element's concentration in the solution. The emitted light is detected by a photocell, which converts it to a voltage that can be recorded and because Na+ and K+ emit light of different wavelengths (colors), the emission of Na+ and K+ (and thus their concentrations) can be measured separately in the same sample by using appropriate colored filters. Al-Obaidi *et al.* (2012) stated that EDTA titration was used to measure calcium and magnesium levels. This method is obtained by adding 10 ml of the sample solution to a 25 ml conical flask for Ca and Mg determinations. 0.05 mol L1 EDTA solution was diluted by a factor of 1/10 to make a 0.005 mol L1 EDTA solution. To the sample solution, add 20 ml of this diluted EDTA. Add 10 ml ammonia buffer and 1 ml Eriochrome Black T indicator solution to the ammonia buffer. Dilution of a 0.025 mol L1 magnesium chloride solution was made by a factor of 1/10 to make a 0.0025 mol L1 magnesium chloride solution. This 0.0025 mol L1 magnesium chloride solution was titrated into the sample solution until a permanent pink colour appeared. This titration with additional samples ware repeated until concordant results was obtained (titres that agree within 0.1 ml). The moles of EDTA added to the sample solution in total were determined. From your concordant results, calculate the moles of the magnesium chloride solution used in the back titration. The moles of Mg2+ will be equivalent to the moles of excess EDTA based on the titration equation below. Calculate the moles of Ca2+ and Mg2+ that must have been complexed with EDTA by subtracting the excess EDTA from the total moles of EDTA added to the sample, given the Ca2+ and Mg2+: EDTA = 1: 1. The moles of Ca2+ and Mg2+ in the sample solution were represented by this result.

Phosphate levels were evaluated using an atomic absorption spectrophotometer (Baur Bio-Medical Electronics Gmbh, Schweringstr, Germany) according to Bello & Abdu (2011) where 2mls of quail sample was added to 50 ml cornical flask, 2 mls of phosphorus extraction solution was later added into it. 2 mls of ammonium molybdate was added and distill water was also added to make half of the flask, 1 ml of diluted stannous chloride and distil water was added to make 50 mls volume. Take the absorbance with a spectrophotometer machine at 660 wavelengths. The sample was poured into the cubate after rinsing it with the same sample before putting it into the atomic absorption spectrophotometer.

*Experiment two*

A total of 12 male New Zealand rabbits with age range of 7 to 8 months, body weight range of 1.5-2 kg acquired from National Animal Production Research Institute (NAPRI) were used for the study. The rabbits were randomly grouped into 2 groups (A - quail egg group and B - control group). Those in group 'A' were administered 1.9 ml/kg of quail egg orally based on the dose reported by Shin *et al.* (2010) and George & Onwuchekwa (2016) from the first day of surgery to 12 weeks using oral gavage, while those rabbits in group 'B' were not given any supplement. Radiographs were taken at 2, 4, 6, 8, 10 and 12 weeks to ascertain the level of healing. Three rabbits out the 6 in each group were sacrificed at the end of the 6$^{th}$ week in order to examine the bone defect histologically and with the use of computed tomography.

Feed was withdrawn 12 hours prior to surgery. All the surgical procedures were performed under general anaesthesia. The rabbits were anesthetized by intramuscular injection of ketamine hydrochloride (Laborate Pharmaceuticals, India) at a dose rate of 50 mg/kg, diazepam (Sakar Healthcare Pvt. Ltd., India) at a dose rate of 5.0 mg/kg and local infiltration of the area with lignocaine 1mg/kg (Labacalin®, Laborate Pharmaceuticals, India) (Monazzah *et al.,* 2017). The left forelimb of each rabbit was clipped and prepared aseptically with chlorhexidine. The distal 1/3 of the femur was draped and the distal femur was accessed by making a longitudinal skin incision of approximately 3 cm. The skin, subcutaneous tissue, fascia and muscles were temporarily retracted. The periosteum was exposed. A cylindrical trephine drill (3.5 mm) was used to create a 3.5 mm diameter (Plate II) through-and-through defect in the distal lateral epicondyle of the femur in all the 12 rabbits. The muscle incision was closed with

continuous suture pattern using chromic catgut (size 2-0) and the skin was closed with simple interrupted

pattern using nylon (size 2- 0). The surgery was performed under aseptic conditions and no external splint or surgical implants was used.

*Data analysis*

Each quail egg sample's proximate/nutritional content and mineral composition were expressed as the mean and standard error of the mean (SEM). The statistical analysis was performed using descriptive analysis from Microsoft excel, Windows 2007. One-way ANOVA was used to analyze the radiographic healing assessment using IBM SPSS software (IBM SPSS Statistics version 25.0). Data were considered statistically significant when P value was less than 0.05 ($P< 0.05$).

**Results**

Percentage mean and SEM of proximate composition or nutrient content of Japanese quail eggs obtained from this study was 65.50 ± 0.71 % for moisture, 8.50 ± 0.33 % for ash, 1.50±0.09 % for lipid, 4.73± 0.03 % for crude protein, 19.78± 0.32 % as shown in Table 1. The mineral composition obtained from this study was reported as mean and SEM, which was 42.5 ± 0.56 mg for sodium, 80 ± 0.71 mg for potassium, 6.5 ± 0.32 mg for phosphorus, 0.9 ± 0.07 mg for calcium and 1.3 ± 0.03 mg for magnesium as shown in Table 1.

The radiographic scoring assessment data were expressed as median (minimum-maximum) (Table 2). The radiographic images of the defects taken at week 2 and week 4 revealed a clear radiolucent defect with low radiodensity and air at the defect sites in both groups A and B (Plate III), no obvious difference was observed at this stage. At week 6, the rate of mineral opacity within the defect site of the control group was lower than that of the quail egg group. At week 8, the quail egg group had a higher score of 2(2-3) than the control group 1(1-2), no significant difference was observed at this week. At week 10, two rabbits had healed in the quail egg group indicating a better healing rate than the control group where all the rabbit defect were still present. The quail egg group had a score of 4(3-4) and the control group had a score of 2(2-3). A significant difference was observed between the groups ($P < 0.05$). At week 12, complete healing was observed in the quail egg group with a radiographic score of 4(4-4), while the control group had 2 rabbits with an open defect (Plate IV) at the end of the research, with a radiographic scoring method of 3(3-4).

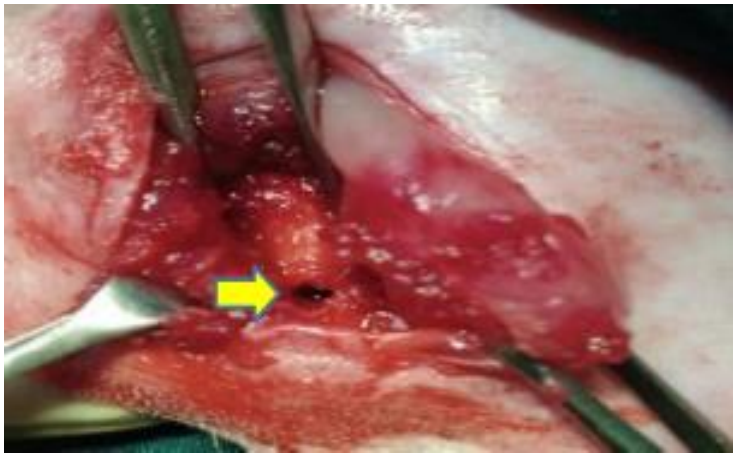

**Plate II**: Defect created on the left distal epicondyle of the femur

**Table 1**: Proximate and mineral composition of Japanese quail egg

| Nutrients (%) | Mean ± SEM |
|---|---|
| Moisture | 65.50 ± 0.71 (%) |
| Ash | 8.50 ± 0.33 (%) |
| Lipid | 1.50 ± 0.09 (%) |
| Crude protein | 4.73 ± 0.03 (%) |
| Carbohydrate | 19.78 ± 0.32 (%) |
| Minerals (mg) | |
| Sodium | 42.5 ± 0.56 mg |
| Potassium | 80.0 ± 0.71 mg |
| Phosphorus | 6.5 ± 0.32 mg |
| Calcium | 0.9 ± 0.07 mg |
| Magnesium | 1.3 ± 0.03 mg |

**Discussion**

A total of 65.5% for moisture was obtained in this study, which was slightly lower than the work reported by Thomas *et al.* (2016), who documented 70% for moisture and also lower that the results of Tunsaringkarn *et al.* (2013) who reported 72.25%, but contrary to the work reported by Ogunwole *et al.* (2015) and Dudusola (2010) who reported much higher values of 78.42 % and 74.26% respectively.

In this study, 8.5% was obtained for ash content, which differs from the findings of Tunsaringkarn *et al.* (2013), Thomas *et al.* (2016) and Dudusola (2010), who obtained much lower values of 1.06 %, 1.07 %, and 1.04 %, respectively. 1.50% was obtained for lipid in this study which was different from that reported by Dudusola (2010) and Tunsaringkarn *et al.* (2013), who reported much higher values of 11.91% and 9.89%, respectively. Weather variations, the nature of the quail feed, and the breed involved could all play a role in the results obtained.

**Table 2**: Radiographic scores of the left femoral bone defect healing in rabbit model administered quail egg

| | Duration after the defect (Weeks) | | | | | |
|---|---|---|---|---|---|---|
| Groups | 2 | 4 | 6 | 8 | 10 | 12 |
| A | 1(1-1) | 1(1-1) | 1(1-1) | 2(2-3) | 4(3-4)[b] | 4(4-4) |
| B | 1(1-1) | 1(1-1) | 1(1-1) | 1(1-2) | 2(2-3) | 3(3-4) |

Data are represented as median (min-max). The latter 'b' indicates a significant difference with the control group ($P < 0.05$)

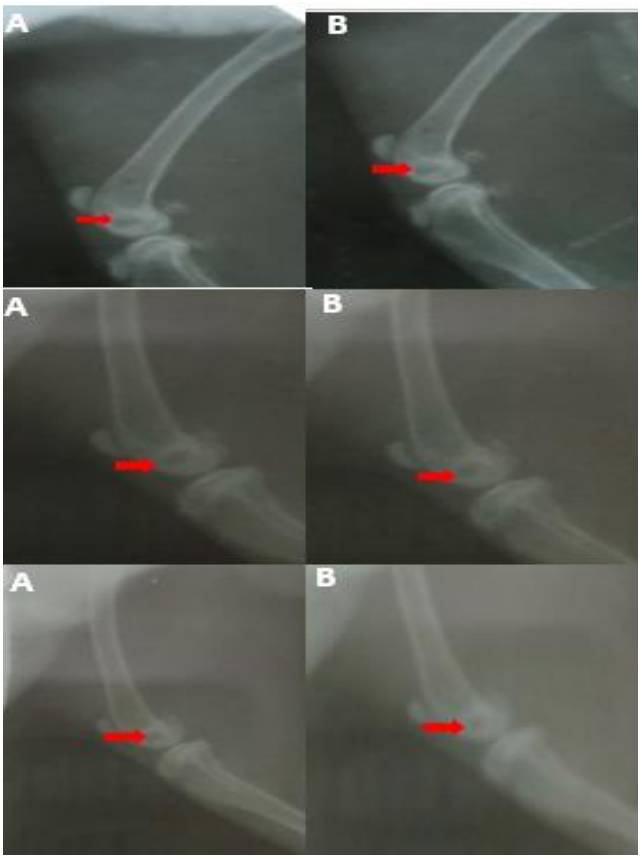


**Plate III:** Radiographs of the left distal epicondyle of the femur at 2, 4, 6 weeks post-operative in the different groups (groups A and B) showing the defect site (red arrow)

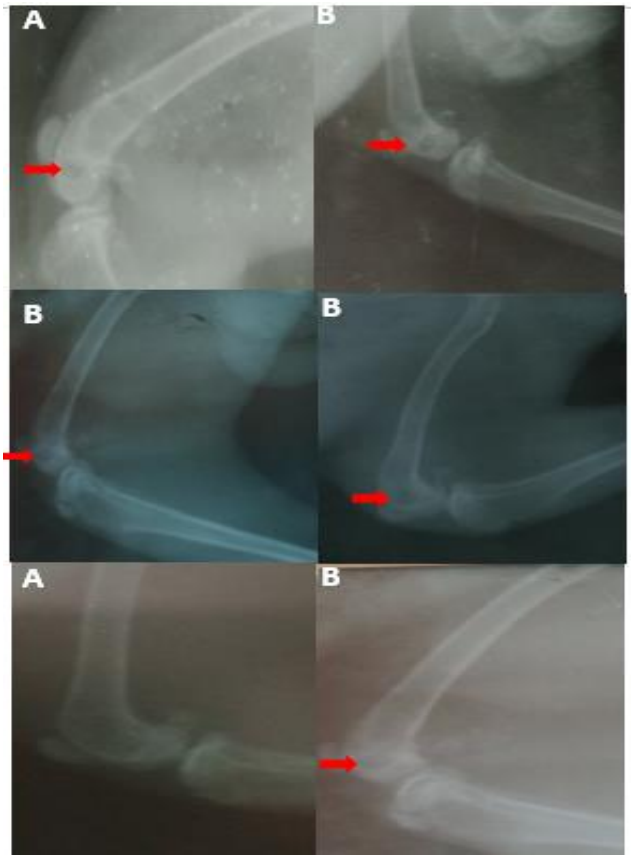


**Plate IV:** Radiographs of the left distal epicondyle of the femur at 8, 10, 12 weeks post-operative in the different groups (groups A and B)

A sum of 4.73% for crude protein was obtained, which is contrary to that reported by Dudusola (2010), Tunsaringkarn *et al.* (2013), and Thomas *et al.* (2016), who reported higher values of 12.7%, 11.98 %, and 13.30 % respectively. Quail eggs are a good source of protein because of their high protein content, and according to Cashman (2007), protein plays an important role in bone healing. In this study, a total of 19.78 % was obtained for carbohydrates, which is similar to the 19.75% obtained by Omoniyi & Abba (2018) after heating for 15 minutes but differs from the lower value of 4.01 % reported by Tunsaringkarn *et al.* (2013). The differences between this study and those reported by Dudusola (2010), Tunsaringkarn *et al*. (2013) and Thomas *et al.* (2016), could be due to breed differences, and atmospheric conditions at the time of lay, and the quail feed given at the time of lay. Mineral elements such as Na, K, Ca, Mg, and P, which are very important in the functioning of the biological system (Ruxton *et al.,* 2016) and can be used as a supplement in bone healing, are one of the various nutrients present in the egg. The high mineral content found in this study is similar to that found by Dudusola (2010), Sarkingobir *et al.* (2020) and Okonko *et al.* (2019) who found quail eggs to be highly nutritious and with balanced minerals. According to Cashman (2007), an adequate calcium dietary intake from quail eggs is a key component of

bone that can significantly reduce bone loss and influence bone healing. This suggests a promising role of nutrition as a nutritional therapy to facilitate bone repair, although extensive research is needed, particularly at the clinical level, to clarify the effectiveness of nutritional interventions in orthopedics. In this study, the mineral content was 42.5 mg for sodium (Na), 80 mg for potassium (K), 6.5 mg for phosphorus (P), 0.9 mg for calcium (Ca), and 1.3 mg for magnesium (Mg). This study found that potassium had the highest value, followed by sodium, which is consistent with Sarkingobir *et al.* (2020), who reported potassium to be higher than sodium, but not with Okonko *et al.* (2019), who found that sodium had the highest value than potassium. After potassium and sodium, phosphorus was the third with a high value (6.5 mg) which was higher than that reported by Okonko *et al.* (2019) but lower than that reported by Sarkingobir *et al.* (2020), who measured 1.98 mg and 8.6 mg, respectively. Mg and Ca were with the least values, with magnesium having higher values than calcium. This pattern obtained was similar to that reported by Okonko *et al.* (2019). When compared with other studies mentioned, the variations in this study could be due to variations in the breed of quails, the nature of feed administered to the quails, and the weather condition at the time of lay.

No work has been documented on the effect of quail eggs on bone defect to the best of our knowledge. The quail egg group had a faster healing rate than the control group, this was in line with the work of Kanczler & Oreffo (2008) who reported that nutrition has a great role to play in hastening the rate of bone healing. The findings of the present study support the popular belief that taking dietary supplement during a healing process facilitates recovery. Bone healing supplemented with quail egg was observed to be significantly higher than the control at week 10, this is in line with the report by Oviawe *et al.* (2020), who found out that excisional wound healed faster in rabbits when quail egg was topically applied. This could be due to the healing property of quail eggs due to its high nutritious content. The bone defect in the control group was not completely healed in 2 of the rabbits at 12 weeks post-surgery which may be attributed to the absence of supplement to accelerate the healing rate of the bone defect.

In conclusion, this study reveals that quail eggs are very nutritious and rich in mineral contents. They are commercially available, inexpensive and therefore could be promising when the whole egg is given as a nutritional supplement during bone healing in order to increase the speed of the healing process.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

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