Rovedar

fahn

Farm Animal Health and Nutrition. 2023; 2(2): 24-29.

DOI: 10.58803/fahn.v2i2.17

http://fahn.rovedar.com/

Research Article

Radiographic Evaluation of Bone Healing Following Oral Administration of Bone Broth and Quail Eggs on Experimentally Drilled Femoral Bone Defect in Rabbits

Ekaete Ime Oviawe[1,2,*], Samuel Tanko Fadasan[2], Maruf Lawal[2], Mohammed Hadi Suleiman[3], and Abdulaziz Abdullahi Bada[2]

[1] Department of Veterinary Surgery and Radiology, Usmanu Danfodiyo University Sokoto, Nigeria
[2] Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Nigeria
[3] Department of Veterinary Anatomy, Ahmadu Bello University, Zaria, Nigeria

* ***Corresponding author:*** Ekaete Ime Oviawe, Department of Veterinary Surgery and Radiology, Usmanu Danfodiyo University Sokoto, Nigeria. Email: kaeteimnoh@gmail.com

ARTICLE INFO

Article History:
Received: 21/04/2023
Accepted: 22/05/2023

check for updates

Keywords:
Bone broth
Bone defect
Bone healing
Quail eggs
Rabbit model
Radiography

ABSTRACT

Introduction: Radiography can monitor bone healing to detect delayed healing, non-union, and mal-union early. This study aimed to monitor bone healing, following oral administration of quail eggs and bone broth (BB) on a bone defect in the rabbit model.
Materials and methods: A total of 24 adult white New Zealand rabbits were used in the study. They were divided into four six groups before creating a 3.5 mm bone defect. The first group received a daily oral dose of BB, the second group a daily oral dose of quail egg (QE), and the third group received a daily oral dose combination of BB and quail eggs (BQE). At the same time, the fourth group was given an oral daily dose of distilled water (CN) for 12 weeks. Radiographs were taken at 2, 4, 6, 8, 10, and 12 weeks postoperative. An experienced radiologist blinded to the groupings scored the radiographs on a scale of 0-4 based on mineral opacity.
Results: At week 6, the BB and BQE groups differed significantly from the QE and CN groups. There was a significant difference between the treatments and the control group at weeks 8 and 10. The complete healing of BB and BQE groups occurred before week 10. The healing of two rabbits in the QE group was done before week 10 although the others completed healing before week 12. The CN group did not heal even after week 12.
Conclusion: Using radiography, Monitoring the bone healing rate was done successfully. The BQE group showed the fastest healing, followed by BB, and QE groups.

1. Introduction

Radiography is an imaging technique that uses X-rays or similar ionizing radiation to view the internal structure of an object[1]. Bone defects are serious conditions in which a portion of the bone is damaged or missing due to trauma, infection, tumors, or congenital malformations. Conventional treatments such as reset, fixation, and functional exercise usually result in complete healing for short bone defects. However, the situation becomes extremely difficult when the defect is too large[2]. Quail egg has several nutritional benefits, such as antioxidants, protein, lipids, cholesterol, carbohydrate, fat, vitamin E, and minerals such as nitrogen, iron, and zinc[3]. Bone broth is traditionally known to improve healing[4]. It is a liquid containing cooked bones and connective tissues, which can be made from cow, chicken, and even fish bones. It is usually simmered for 24 - 48 hours when cooking, giving the stock enough time to extract all the nutrients from the bones[5]. It has been estimated that approximately 5–10% of the population experience difficulty in bone healing, resulting in delayed bone healing, non-unions, and malunions, which could have been avoided with adequate monitoring and nutritional support[6]. Bone healing is a process of renewal of the bone tissue[7]. The ability of bone tissues to repair itself often fails when a defect is too complicated or too large to be bridged, often leading to delayed healing[8]. Surgical management and autografts are the current gold standard treatment for bone defects, which can be used along with adjunct therapy to enhance bone healing[9,10]. Thus, the high costs associated

► *Cite this paper as Oviawe EI*, Fadasan ST, Lawal M, Suleiman MH, Abdullahi Bada A. Radiographic Evaluation of Bone Healing Following Oral Administration of Bone Broth and Quail Eggs on Experimentally Drilled Femoral Bone Defect in Rabbits. Farm Animal Health and Nutrition. 2023; 2(2): 24-29. DOI: 10.58803/fahn.v2i2.17

with a high incidence of delayed bone healing and complications place considerable financial, physical, and mental burdens on individuals and highlight the need to identify affordable, effective, and safe strategies to accelerate the healing rate, especially in a stable bone defect.

2. Material and Methods

2.1. Ethical approval

Ahmadu Bello University (Nigeria) Committee approved this research on animal Use and Care (ABUCAUC). An approval number (ABUCAUC/2021/096) was issued.

2.2. Animals and study design

Twenty-four males aged 7-8 months New Zeeland rabbits weighing 1.7 ± 0.28 kg obtained from the National Animal Production Research Institute (NAPRI, Zaria, Nigeria) were used for this study in August 2021. On the day of the surgical creation of the femoral bone defect, the twenty-four rabbits were randomly grouped into four groups (A[BB], B[QE], C[BQE], and D[CN]) of six animals each. The rabbits in group A were orally given 3 ml/kg of bone broth (BB). Group B received 1.9 ml/kg of quail egg (QE) orally, while group C was given a combination of bone broth (3 ml/kg) and quail egg (1.9 ml/kg, BQE) orally[11,12,13]. In group D, rabbits were not given any supplement (CN) and only received an oral daily dose of distilled water. All the treatments were administered daily from day 1 of surgery till week 12. The animal preparation, anesthesia used, and surgical creation were performed[14]. This procedure was performed in all 24 rabbits. Aseptic conditions were adhered to, and no external splint or surgical fixation was used[15]. No post-surgical complication was observed. A mediolateral radiograph of the left hind limb of each rabbit was taken at weeks 2, 4, 6, 8, 10, and 12 postoperative till the end of the study using a mobile x-ray machine (MDX 100, Records and Medicare Systems (P) Ltd. India). Radiographic evidence of healing included a progressive increase in mineral opacity in the defect site. An experienced radiologist blinded to the groupings on the work scored all the radiographs on a scale of 0–4 using a previously published semi-quantitative scoring system with slight modification[16]. Including scale 0, no mineral opacity in the defect area; scale 1, minimal mineral opacity of 0 > 25% in the defect site; scale 2, mineral opacity of 25>50% in the defect site; scale 3, mineral opacity of 50>75% in the defect site, scale 4, mineral opacity of 75>100% bridging bone at the defect sites.

2.3. Statistical analysis

The statistical analyses were performed using IBM SPSS software (IBM SPSS Statistics version 25.0, USA). Radiographic data were presented as median (minimum-maximum) values. The Kruskal-Wallis test was used to analyze the radiographic results. Data were considered statistically significant when $p < 0.05$.

3. Results

A radiographic assessment of bone healing revealed a clear radiolucent defect site in the left distal epicondyle of the femur (Table 1). There was no significant difference ($p > 0.05$) in the radiologic scores among the groups at weeks 2 and 4 (Table 1, Figure 1, and Figure 2). However, a

Table 1. Radiographic scores of the left distal femoral bone defect healing in rabbit models were administered with quail egg and bone broth in Nigeria in August 2021

Groups	Duration of bone healing after the bone defect					
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
BB	1(1-1)	1(1-1)	1(1-2)*	3(2-3)*	4(4-4)*	4(4-4)
QE	1(1-1)	1(1-1)	1(1-1)	2(2-3)*	4(3-4)*	4(4-4)
BQE	1(1-1)	1(1-1)	2(1-2)*	3(3-3)*	4(4-4)*	4(4-4)
CN	1(1-1)	1(1-1)	1(1-1)	1(1-2)	2(2-3)	3(3-4)

Data are represented as median (range). *asterisk within each week (within the column) indicates a significant difference ($p < 0.05$) among the groups. 0-6 weeks (n=6), 6-12 weeks (n=3). BB: Bone broth group, QE: Quail eggs group, BQE: Bone broth and quail egg combination group, CN: Control group

Figure 1. The rabbit model's left distal femoral epicondyle bone defect after two weeks of treatment. Round radiolucent defect site (yellow arrow) across the groups in Nigeria. BB: Bone broth, QE: Quail egg, BQE: Bone broth and quail egg combination, CN: Control

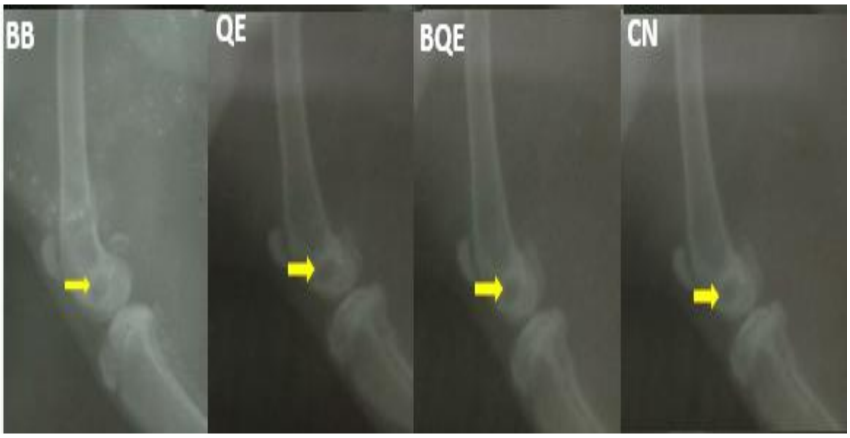

Figure 2. The rabbit model's left distal femoral epicondyle bone defect after four weeks of treatment. Round radiolucent defect site (yellow arrow) across the groups in Nigeria. BB: Bone broth, QE: Quail egg, BQE: Bone broth and quail egg combination, CN: Control

statistically significant difference was observed among the treatment groups of bone broth (BB) group, bone broth and quail eggs combination (BQE) group with respect to the control (CN) group at week 6 ($p < 0.05$). From the radiographs, at week 6 it was observed that the shape and dimension of the defect site changed on the radiographs (Figure 3) of BB and BQE, indicating that the reparative phase has ended and the healing was currently in its final stage (remodeling phase) while the quail eggs (QE) and CN group were still in the reparative phase. A statistically significant difference in the radiologic scores were also observed among all the treatment groups (BB, QE, and BQE) with respect to the CN group at week 8 and 10 ($p < 0.05$). The BB and BQE were in their early remodeling phase (third phase), while QE and CN were in their late reparative phase (second phase, Figure 4) at week 8. Complete healing and remodeling of the bone defect in the BB and BQE

Figure 3. The rabbit model's left distal femoral epicondyle bone defect after six weeks of treatment. The radiolucent defect site (yellow arrow) showed changes in its dimension and was shaped with more callus deposits (radiodensity) around the defect site observed in BB and BQE groups in Nigeria. BB: Bone broth, QE: Quail egg, BQE: Bone broth and quail egg combination, CN: Control

Figure 4. The rabbit model's left distal femoral epicondyle bone defect after eight weeks of treatment. The radiolucent defect site (yellow arrow) showed changes in dimension and shaped with more callus deposits (radiodensity) around the defect site observed in all the groups in Nigeria. BB: Bone broth, QE: Quail egg, BQE: Bone broth and quail egg combination, CN: Control

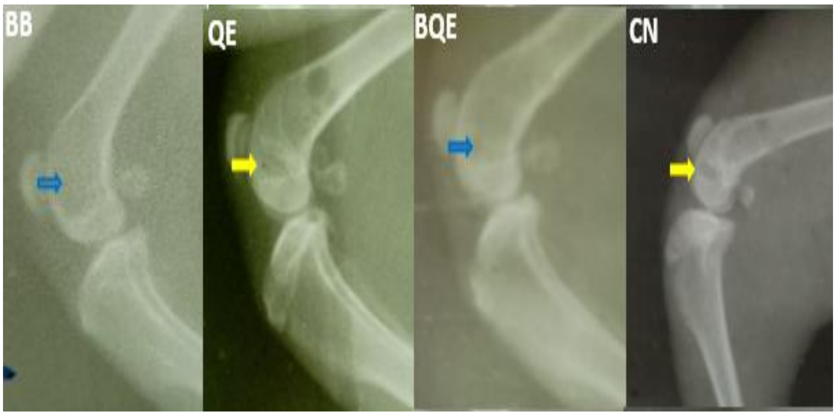

Figure 5. The rabbit model's left distal femoral epicondyle bone defect after 10 weeks of treatment. The disappearance of the radiolucent defect in the BB and BQE group (blue arrow) was still present in QE and CN groups (yellow arrow) in Nigeria. BB: Bone broth, QE: Quail egg, BQE: Bone broth and quail egg combination, CN: Control

group at week 10 (Figure 5) and QE group at week 12 (Figure 6) was observed. While in the CN group, there was evidence of woven bone at the center of the defect without complete bone healing and remodeling.

Figure 6. The rabbit model's left distal femoral epicondyle bone defect after 12 weeks of treatment. Complete healing of the defect was observed in all the treatment groups (BB, QE, and BQE, blue arrows) and incomplete with a radiolucent defect (yellow arrow) in the CN group in Nigeria. BB: Bone broth, QE: Quail egg, BQE: Bone broth and quail egg combination, CN: Control

4. Discussion

The radiographs taken at 2 weeks intervals revealed faster progressive healing in all the treatment groups compared to the control group. In addition, it was discovered that the BB and BQE group healed much faster than the QE and CN groups. In this study, the earliest observed changes were blurring of the defect margins with changes in the shape of the defect site at week 6. Based on the radiographic scoring, no significant difference was observed until week 6. This was likely because the radiograph did not pick the early signs of healing, which was in progress. This finding is similar to the work reported by a study, which worked on calcium supplements in rats and reported no significant difference at weeks 2-4[17]. The reparative phase was observed to have occurred before week 2, which ended before week 6 for BB and BQE groups and ended before week 8 for QE and CN groups, after which the remodeling stage started. The changes in the shape and dimension of the defect site and the hard callus indicate that the reparative phase is over. This finding supports the report of an examination that documented that the structure of the fractured site differs from its original look at the end of the repair phase[18]. At week 6, the treatment groups significantly increased the radiographic score, especially among the BB and BQE groups, compared to the QE and CN groups. This is likely because bone broth contains high calcium, protein, and other essential nutrient levels for bone healing. This finding aligns with the work that administered calcium supplements on fracture in an osteoporotic rat model and observed significant changes in healing at week 6[17]. This finding also agrees with the work that reported a significant difference at the end of the fifth week in the healing of fractured bone in rabbits due to daily oral administration of bone broth supplement[11]. This finding is also in line with the work that assessed the effects of combined calcium hydroxyapatite crystals and human platelet-rich plasma on critical-sized bone defects of the radius in a rabbit and reported a significant difference from week six between the treatment groups and the control[19]. At week 8 and week 10, the significant difference obtained between the treatment groups when compared to the control group was likely because all the nutrients such as crude protein, carbohydrate, calcium, phosphorus, potassium, sodium, and magnesium obtained in the bone broth and quail eggs must have contributed in enhancing the healing process in the treatment groups. This finding agrees with the work reported on the advances and promises of nutritional influences on natural bone repair and the work on the nutritional aspects of bone health and fracture healing[20,21]. The bone broth has a high nutritional content, including calcium and protein, all required for bone regeneration. This study's findings support the widely held assumption that taking a dietary supplement throughout the healing phase enhances recovery. The CN group was delayed because there was no nutrition in the distilled water to expedite bone defect repair. The healing time of 10 weeks obtained in the BB and BQE group is contrary to the healing time that worked on bone broth and reported a healing time of a transverse fracture at the 5th week[19]. The variations in the healing time are because this study created a 3.5 mm defect, not a transverse fracture.

5. Conclusion

Radiography monitored the rate of bone healing across the groups and revealed that the treatment groups healed faster than the control group. The BQE and BB groups showed the best results in bone healing. This research can be repeated in clinical cases and on fractured bones in the future. It can also be conducted on different sex groups to know if hormonal changes can affect the healing process.

Declarations

Competing interests

The authors declare that they have no conflict of interest or personal relationships that could have influenced the work done in this paper.

Authors' contributions

Ekaete Ime Oviawe and Samuel Tanko Fadasan designed the study. Ekaete Ime Oviawe and Lawal Maruf wrote the manuscript. Sunday Tanko Fadasan and Maruf Lawal provide materials, patients, and resources. Mohammed Hadi Suleiman and Abdulaziz Abdullahi Bada were involved in data analysis and manuscript revision. The final manuscript draft was reviewed by all authors, who approved it.

Funding

TetFund sponsored the research work.

Availability of data and materials

The authors will provide all necessary data to the editor upon request.

Ethical considerations

All authors ensured that ethical issues such as plagiarism, consent to publish, misconduct, fabricated and falsified data, dual publishing and submission, and redundancy were examined.

Acknowledgments

None.

References

1. Chen T, Lai RF, and Zhou ZY. Application of ultrasonic inspection in monitoring dynamic healing of mandibular fracture in rabbit model. Asian Pac J Trop Med. 2012; 5(5): 406-409. DOI: 10.1016/s1995-7645(12)60068-6
2. Mahmood SK, Razak IS, Ibrahim SM, Yusof LM, Abubakar AA, Mahmood ZK et al. *In Vivo* Evaluation of the novel nanocomposite porous 3D scaffold in a rabbit model. Indian Journal of Science and Technology. 2018; 11(19): 1-15. DOI: 10.17485/ijst/2018/v11i19/122540
3. Thomas K, Jagatheesan PN, Reetha T, and Rajendran D. Nutrient Composition of Japanese Quail Eggs. Int J Sci Environ Technol. 2016;5:1293-95.
4. Lieberman S. The real vitamins and minerals book. 2nd ed. New York: Avery publishing company; 1997. p. 162-195.
5. Schauss A, Stenehjem J, Park J, Endres J, and Clewell A. Effect of the novel low molecular weight hydrolyzed chicken sternal cartilage extract, BioCell Collagen, on improving osteoarthritis-related symptoms: A randomized, double-blind, placebo-controlled trial. J Agric Food Chem. 2012; 60(16): 4096-4101. DOI: 10.1021/jf205295u
6. Zura R, Xiong Z, Einhorn T, Watson JT, Ostrum RF, Prayson MJ et al. Epidemiology of fracture non-union in 18 human bones. JAMA Surg. 2016; 151(11): e162775. DOI: 10.1001/jamasurg.2016.2775
7. Blumenfeld I, Srouji S, Lanir Y, Laufer D, and Livne E. Enhancement of bone defect healing in old rats by TGF-b and IGF-1. Exp Gerontol. 2002; 37(4): 553-565. DOI: 10.1016/s0531-5565(01)00215-7
8. Janicki P, and Schmidmaier G. What should be the characteristics of the ideal bone graft substitute? Combining scaffolds with growth factors and/or stem cells. Injury. 2011; 42(Suppl 2): S77-S81. DOI: 10.1016/j.injury.2011.06.014
9. Liu G, Zhao L, Zhang W, Cui L, Liu W, and Cao Y. Repair of goat tibial defects with bone marrow stromal cells and betatricalcium phosphate. J Mater Sci Mater Med. 2008; 19: 2367-2376. DOI: 10.1007/s10856-007-3348-3
10. Theos CKP, Koulouvaris P, Kottakis S, and Demertzis N. Reconstruction of tibia defects by ipsilateral vascularized fibula transposition. Arch Orthop Trauma Surg Neurol. 2008; 128(2): 179-184. DOI: 10.1007/s00402-007-0301-3
11. Aljumaily MA. The effect of concentrated bone broth as a dietary supplementation on bone healing in rabbits. Ann Coll Med Mosu. 2011; 37(1): 42-47. DOI: 10.33899/mmed.2011.34633
12. Shin JW, Seol C, and Son CG. Interpretation of animal dose and human equivalent dose for drug development. Korean J Orient Med. 2010; 31(3): 1-7. Available at: https://www.jkom.org/upload/31-3%2001%20[01-07].pdf
13. George GO, and Onwuchekwa TT. Effect of raw quail egg on intraocular pressure and blood pressure of hypertensive subjects. J adv med pharm sci. 2017; 11(3): 1-9. DOI: 10.9734/JAMPS/2016/29537
14. Oviawe EI, Fadason ST, Lawal M, and Suleiman MH. Proximate and mineral composition of Japanese quail egg and its possible role in bone healing. Sokoto J Vet Sci. 2022; 20(2): 111-118. DOI: 10.4314/sokjvs.v20i2.5
15. Rumi MN, Deol GS, Singapuri KP, and Pellegrini VD. The origin of osteoprogenitor cells responsible for heterotopic ossification following hip surgery: An animal model in the rabbit. J Orthop Res.2005; 23(1): 34-40. DOI: 10.1016/j.orthres.2004.05.003
16. Minto BW, Neto GJ, Sprada AG, Quarterone C, Sartori MR, Alcântara, BM et al. A critical size defect model in the radius of rabbits. Ars

Veterinaria. 2020; 36(3): 187-194. DOI: 10.15361/2175-0106.2020v36n3p187-194

17. Shuid AN, Mohamad S, Mohamed N, Fadzilah FM, Mokhtar SA, Abdullah S et al. Effects of calcium supplements on fracture healing in a rat osteoporotic model. J Orthop Res. 2010; 28(12): 1651-1656. DOI: 10.1002/jor.21180
18. Bigham-Sadegh A, and Oryan A. Selection of animal models for pre-clinical strategies in evaluating the fracture healing, bone graft substitutes and bone tissue regeneration and engineering. Connect Tissue Res. 2015; 56(3): 175-194. DOI: 10.3109/03008207.2015.1027341
19. Oryan A, Parizi AM, Shafiei-Sarvestani Z, and Bigham AS. Effects of combined hydroxyapatite and human platelet-rich plasma on bone healing in rabbit model: Radiological, macroscopical, hidtopathological and biomechanical evaluation. Cell Tissue Bank. 2012; 13(4): 639-651. DOI: 10.1007/s10561-011-9285-x
20. Robert JL, and Drissi H. Advances and promises of nutritional influences on natural bone repair. J Orthop Res. 2020; 38(4): 695-707. DOI: 10.1002/jor.24527
21. Karpouzos A, Diamantis E, Farmaki P, Savvanis S, and Troupis T. Nutritional aspects of bone health and fracture healing. J Osteoporos. 2017; 2017: 4218472. DOI: 10.1155%2F2017%2F4218472