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Analysis of the Mechanical Properties of Femurs and Eggshells of Two Selected Japanese Quail Lines Under Quasi-Static and Impact Loading Conditions

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ABSTRACT

This study presents the results of a dynamic two-point bending test and a quasi-static three-point bending test of the quail femurs. Bones from females and males of two genetic strains of Japanese quail belonging to various utility types were analyzed. Mechanical parameters obtained under impact and quasi-static loading conditions were investigated. The mechanical strength of eggshells under both loading conditions was also examined. The obtained results showed that the bones of males of both analyzed types of quails were characterized by statistically significant higher strength obtained under impact loading conditions compared to the bones of females. Moreover, the mechanical strength of the eggshell measured under impact loading conditions was characterized by higher values compared to the result obtained under quasi-static load conditions. The observed differences between quail genetic lines were not statistically significant. SEM–EDS qualitative elemental distribution analysis showed a higher content of calcium in the female femurs. The damage present on the fracture surface indicates that these bones were more brittle.

Keywords: bone fracture, bone strength, eggshell strength, impact loading condition, quasi-static three-point bending test.

INTRODUCTION

A bird's skeleton provides structural support and is a source of minerals for the eggshell formation in female birds. It is specialized for flying and walking on two legs. In many animals, cortical and trabecular bone thickness decreases with age. The bones of laying birds also change overall strength throughout their lives. The mechanical strength of bones depends on geometrical properties, the degree of mineralization, and the quality of the material from which they are built. Due to the participation of individual elements (cells, organic matrix, inorganic substances), their spatial organization, and the functions performed, three types of bone tissue have been distinguished in birds: cortical, trabecular, and medullary bone - occurring only in mature females [1–3]. A medullary bone, as specialized easily created, and resorbed woven bone, serves as a calcium reservoir in the process of eggshell formation. However, if a bird is calcium deficient, it can also use cortical and trabecular bone as a source. During lay, the content of the medullary bone increases while the structural bone integrity decreases. The loss of structural bone is not compensated by the mechanically weaker medullary bone formation. This skeletal weakening is considered to be a type of osteoporosis [4–7].

Japanese quails (*Coturnix japonica*) are valuable birds for research because of their small size and rapid growth rate. The Japanese quail undergoes an aging process similar to that of mammals with deterioration of reproductive functions, metabolic and sensory systems [8]. The characteristics of their bones may be determined by genetic, physiological, nutritional, and physical factors [6, 9]. Bones, despite their great hardness, exhibit a certain elasticity and mechanical plasticity and also react with a change in structure to the continuous or repeated action of deforming forces related to loading and unloading. From a mechanical point of view, bone tissue is a unique material, the characteristics of which are determined by its structure as well as the crystallinity of the bone mineral [10].

The eggshell strength is the most important issue during the handling of packaged food. Eggshell breaking may occur under quasi-static loading conditions e.g. throughout the storage in packaging trays. But the greatest part of the damage is due to the forces acting under impact loading conditions during oviposition, rolling out of the cage, hitting other eggs in the grading process, etc. [11, 12]. The eggshell rupture force may depend on the poultry breed, diet, supplementation, egg shape, eggshell microstructure and topography as well as loading velocity [11, 13, 14]. The most common technique used for eggshell strength determination is a compression test between two plane plates but this method is limited to using the compression rate up to 5 $\mathrm{mm \cdot s^{-1}}$ [11]. Nedomova et al. [15] used an impact test of a freely fall bar from different heights to evaluation of an eggshell's mechanical characteristics. They concluded that the dynamic rupture force was higher than that obtained at the static loading. Trnka et al. [11] used the Hopkinson split pressure bar technique to analyze the dynamic strength of goose eggs. This method allowed for achieving loading rates up to about 17 $\mathrm{mm \cdot s^{-1}}$. They found that the rupture force obtained at the high strain rate is independent of the eggshell curvature.

In the literature, the bone strength was analyzed using a classical three-point bending test under quasi-static loading conditions [6, 16–18]. However, bone fractures most often occur when a large force acts on the bone in a short period. There is no data describing the quail bone behavior under such mechanical loading conditions. To date, the biomechanical properties of femurs of selected quail lines have not been compared. There is also no data on the differences in mechanical properties of males and females of this bird species. This study aimed to determine the bone mechanical parameters of quail femurs measured under quasi-static and impact loading conditions. The effect of genetic line and sex on the studied parameters was examined. This work used a scanning electron microscopy technique to provide information about bone structure and chemical composition which have important contributions to bone mechanical properties. Additionally, a new approach in the eggshell strength determination was applied.

MATERIALS AND METHODS

The research material was obtained from Japanese (*Coturnix coturnix japonica*) quails, following their intentional slaughter at the end of their reproductive utility. Femurs of two genetic strains F11 (meat type) and S22 (lying type) were used in the study. In accordance with the "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes," it is not permissible to euthanize an animal solely for the purpose of utilizing its organs or tissues for the specified purposes in the directive". Consequently, the approval of the ethics committee was not obtained.

The birds were maintained for a period of 24 weeks in reproductive flocks (1 ♂ × 4 ♀) under conditions compliant with prevailing legislation, adhering to a lighting program (16L:8D), and fed standard feed mixture adjusted to the age and physiological state of the birds. At the age of 24 weeks, 36 eggs were collected from birds of each breed. Right and left femurs were dissected after the birds were sacrificed. 10 right and left bones from females and males of each breed were collected. The bones were frozen and stored at -20 ºC until strength tests were carried out. Bone length and eggshell thickness were measured by an electronic micrometer. Bone thickness was determined after mechanical tests based on microscopic measurements (Figure 1) (Nikon SMZ18, Nikon Corporation, Tokyo, Japan).

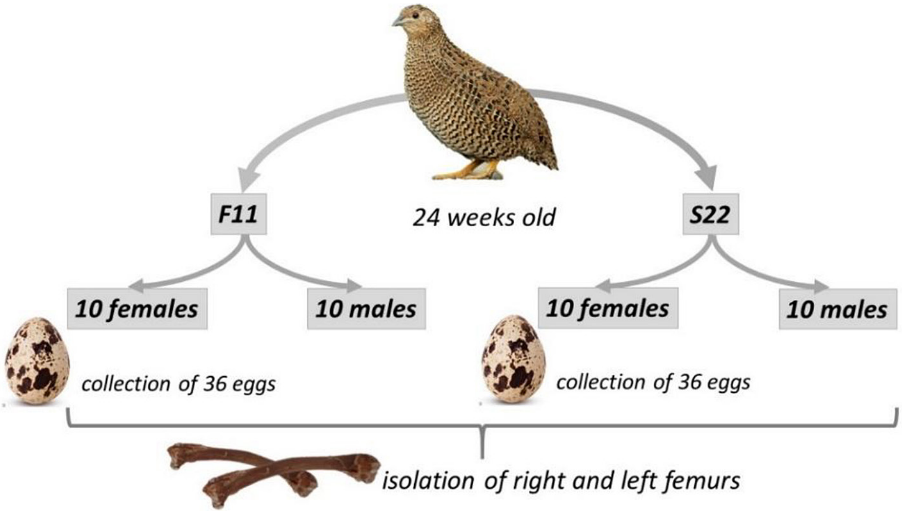


Figure 1. Scheme of the experiment

Strength analysis under quasi-static loading condition

Bone mechanical properties were determined from the force-deformation curve recorded in a three-point bending test using a TA.HD plus texture analyzer (Stable Micro System, Godalming, UK). The average femurs length was 40.18 ± 1.00 mm, therefore the distance between supports (*L*) was set at 16 mm (40% of the total bone length). The measuring head speed was constant at 10 mm·min^{-1}. The tests were performed at a sampling frequency of 100 Hz.

Three-point bending test provided the determination of bone strength as a maximum force recorded under quasi-static loading condition, work-to-fracture, stiffness, maximum stress and modulus of elasticity. For the calculation it was assumed that the bone cross-section was in the shape of an ellipse [18]. Shell strength was analyzed using an Instron Mini 55® (American Instrument Exchange, Haverhill, MA, USA) strength testing apparatus. The force required to fracture the eggshell continuity was measured with the head speed at 50 mm·min^{-1}.

Impact test

In order to perform the bone impact test the methodology described in [19] was applied. Males and females femurs were isolated and subjected to two-bending test under impact loading condition. Bones were immobilized in the epoxy glue-filled PVC tube as presented in Figure 2. The height of the sample measured from the tube edge to the femur head was set to 17 mm. The impact test was made at a constant speed of $V_1 = 0.5$ m·s^{-1}, with the moment of inertia of the pendulum arm $I = 0.072$ kg·m^2. The impact force (as a bone strength under impact loading conditions) was measured by piezoelectric force sensor

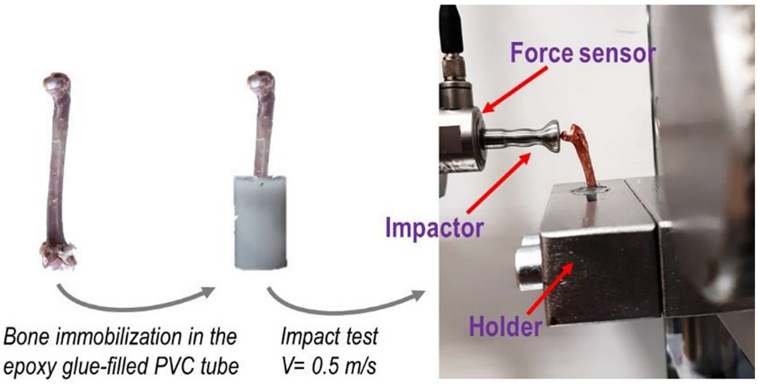


Figure 2. Test stand for dynamic testing of quail femurs

Endevco model 2311–100 (Endevco Corporation, Sunnyvale, CA, USA) of 2.25 mV·N^{-1} sensitivity and measurement range ± 220 N.

In order to carry out the eggshell impact test, a specially designed holder was used and mounted to the previously described device. Therefore, a shaped form, made of flexible silicone compound, which can be freely shaped in a plastic form, has been used in these tests. Geometrical parameters of the eggs were measured and their average sizes determined before constructing the test holder. These concerned the overall dimensions, i.e. length of the egg and horizontal diameters measured relative to two mutually perpendicular axes (Fig. 3).

Taking into consideration the geometric parameters of the modeled surface, an egg holder design was developed. The egg holder was made of medium hardness flexible silicone rubber, POLASTOSIL M-33, and OL-1 catalyst manufactured by "Silikony Polskie" Sp. z o.o. Nowa Sarzyna (Poland). The density of the mass provided an adequate strength base to support tested surface. The tested egg was placed in prepared deformable form. The holder with egg, comprising the testing set, were mounted on the main board of the testing stand, as shown in Figure 4. 18 eggs from each group (F11 and S22) were taken for testing. Eggs were placed in a previously prepared mold and the flat plate impactor hit the eggshell at a constant speed of $V_2 = 0.25\ \text{m}\cdot\text{s}^{-1}$.

Microstructure analysis

Quail femurs after mechanical testing were taken to fracture surface imaging and qualitative elemental distribution analysis using the Phenom ProX scanning electron microscope (Thermo Fisher Scientific Inc., Waltham, MA, USA). The bones were imaged without pre-treatment with the 10 kV accelerating voltage. The elemental distribution analysis was made using energy dispersive spectroscopy (EDS system) with a voltage of 15 kV. Maps of elemental concentration were taken from the area of 250×250 µm in triplicates. Based on obtained data Ca/P ratios were calculated.

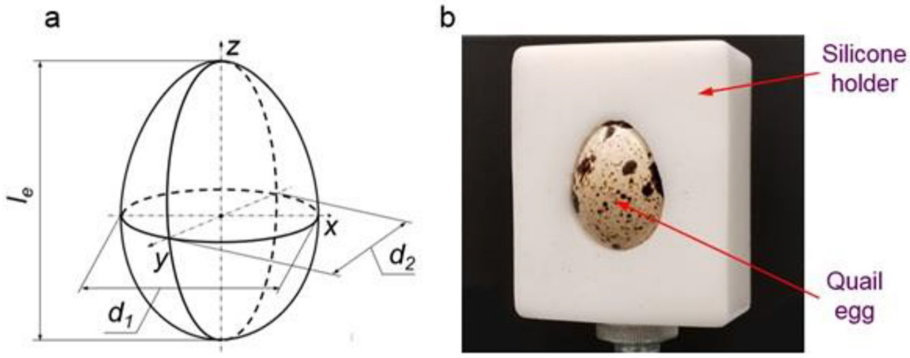


Figure 3. Construction of the quail egg test set (TS), a – geometrical parameters of egg: le – length of the egg, d_1 – horizontal diameter along the x axis, d_2 – horizontal diameter along the y axis, b – the silicone holder with egg – the test set (TS)

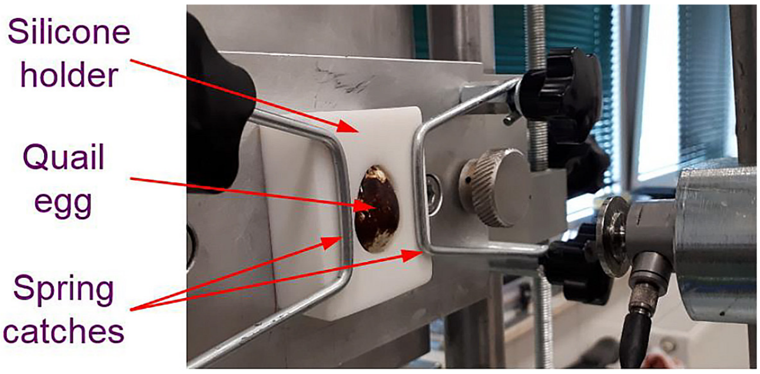


Figure 4. The position of the testing set (TS) in the measuring stand

Statistical analysis

Statistical data analysis was performed in the STATISTICA 13.1 (StatSoft, Inc., Tulsa, OK, USA). The basic descriptive statistics (mean ± standard deviation) of each parameter were calculated. Two-way analysis of variance (ANOVA) was used to compare bone and eggshell properties between quail groups. Differences between groups were determined with Tukey's test. Two factors were considered in analyses: quail breed and sex. A $p \leq 0.05$ value was considered as significant.

RESULTS

Table 1 summarizes the main characteristics of quails, isolated femurs and eggs. Females had a greater body weight than males and had a greater average femur length. The isolated bones had a similar thickness ranging from 0.32 ± 0.09 mm to 0.35 ± 0.06 mm. Quail eggs of the S22 line had a greater mass and slightly greater shell thickness compared to those of the F11 line. The observed differences in the mean values of these parameters were not statistically significant. The results

Table 1. Characteristics of analyzed quails, bones and eggs. Given mean values are presented with standard deviations. Statistically significant differences between groups (Tukey´s tests) are indicated by a, b, c

Parameters	F11		S22	
	Male	Female	Male	Female
Body weight (g)	178.20 ± 8.02^{b}	203.25 ± 14.73^{a}	159.70 ± 11.08^{c}	194.83 ± 11.97^{a}
Femur length (mm)	40.29 ± 0.87^{a}	41.10 ± 0.55^{a}	39.41 ± 0.17^{a}	39.91 ± 1.19^{a}
Femur thickness (mm)	0.35 ± 0.06^{a}	0.32 ± 0.09^{a}	0.33 ± 0.12^{a}	0.33 ± 0.08^{a}
Egg weight (g)		10.50 ± 0.29^{a}		10.94 ± 0.33^{a}
Eggshell thickness (mm)		0.24 ± 0.02^{a}		0.25 ± 0.02^{a}

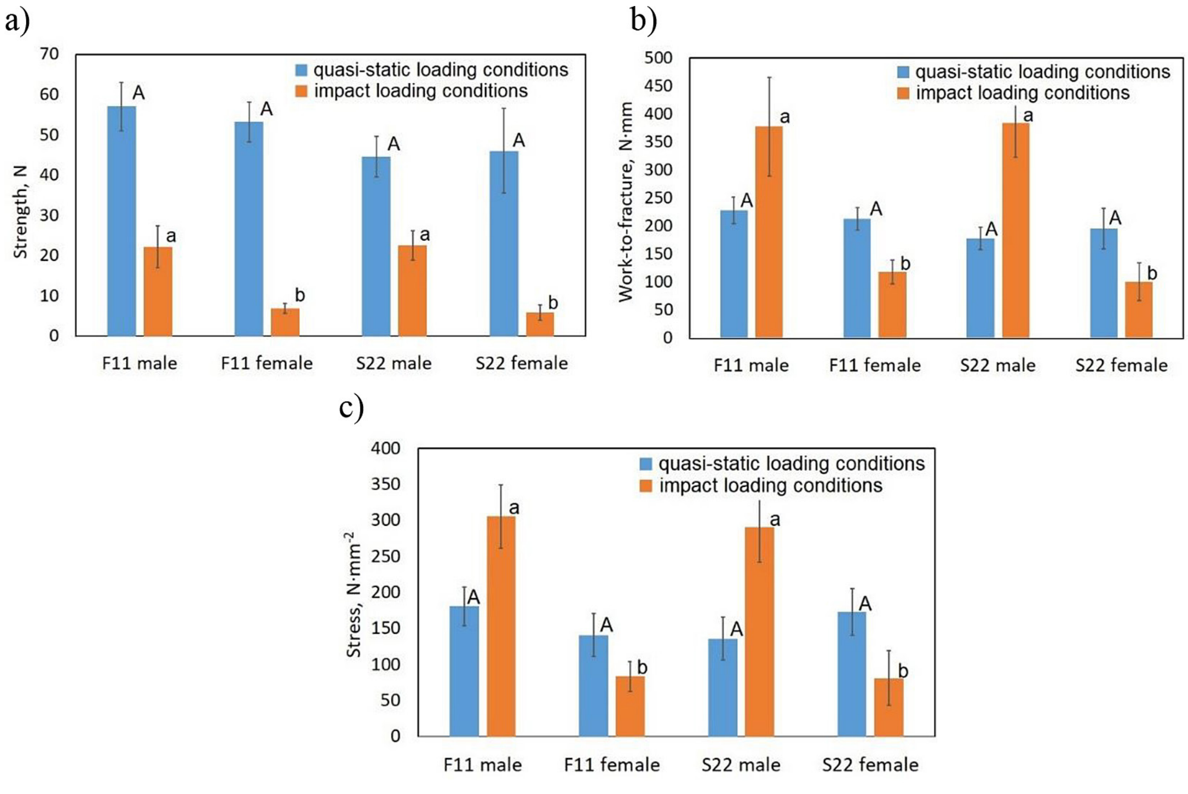


Figure 5. Mean values with standard deviations of mechanical parameters obtained under quasi-static (blue bar) and impact loading conditions (orange bar) for males and females lines F11 and S22. The same upper or lower case letters indicate no significant differences between the means under quasi-static and impact loading conditions, respectively

of the mechanical tests are summarized in Figure 5, which shows the strength, work-to-fracture and stress values obtained under quasi-static and impact loading conditions for two quail breeds. The highest force values needed to bone break under quasi-static loading conditions were obtained for the femurs of F11 males (Fig. 5a). Also for this group work-to-fracture (Fig. 5b) and stress (Fig. 5c) were characterized by highest mean values. Calculated stiffness and Young's modulus had higher values for the meat type than for the laying breed of quail. Stiffness was 156.08 ± 14.76 N·mm^{-1} for males and 162.27 ± 13.93 N·mm^{-1} for females of the F11 group and 135.88 ± 14.72 N·mm^{-1} for males and 139.26 ± 22.32 N·mm^{-1} for females of the S22 group. The mean values of Young's modulus in the F11 group were 12.21 ± 3.48 GPa and 13.63 ± 4.56 GPa for males and females respectively and for the S22 line, the values were 8.13 ± 1.81 GPa and 11.28 ± 4.86 GPa in the male and female group. Under impact loading conditions, mean values of all measured mechanical parameters were significantly higher for males than for females (Fig. 5) in both quail genetic lines. The highest values of work-to-fracture and stress were obtained for the F11 males, while the lowest mean values of all measured parameters were observed for S22 females. Analysis of variance showed significant differences in measured mechanical parameters under impact load conditions between females and males femurs but differences between F11 and S22 genetic strains were not statistically significant.

The eggshell strength measured under impact loading conditions had greater values (22.04 ± 2.30 N and 22.41 ± 1.76 N) than those measured under quasi-static conditions (15.07 ± 2.93 N and 16.80 ± 3.35 N for F11 and S22 eggs, respectively). Performed analysis of variance showed no statistically significant differences in eggshell strength between tested quail lines (Fig. 6).

Figure 7 presents microscopic photographs of bone fracture at magnifications 300× (Fig. 7a, c, e, g) and 2000× (Fig. 7b, d, f, h). The fracture surfaces of the male bones were smooth with a straight crack path. In contrast, the fracture surface of the female bones showed multiple cracks and structural damage. These damages indicate that the female bones were more brittle. In Figures 7c and 7g a medullary bone is visible as a porous, rich in calcium structure. Elemental analysis showed a significantly higher calcium content in the female femurs than in those of males (Table 2). Due to similar phosphorus content, the Ca/P ratio was also higher for females. The content of other elements was at a similar level in all analyzed femurs.

DISCUSSION

Suzer et al. [9] studied the effect of different feather colors on quail tibia mechanical properties and showed that under quasi-static loading conditions (head speed of 10 mm·min^{-1}) there were no statistically significant differences in strength and

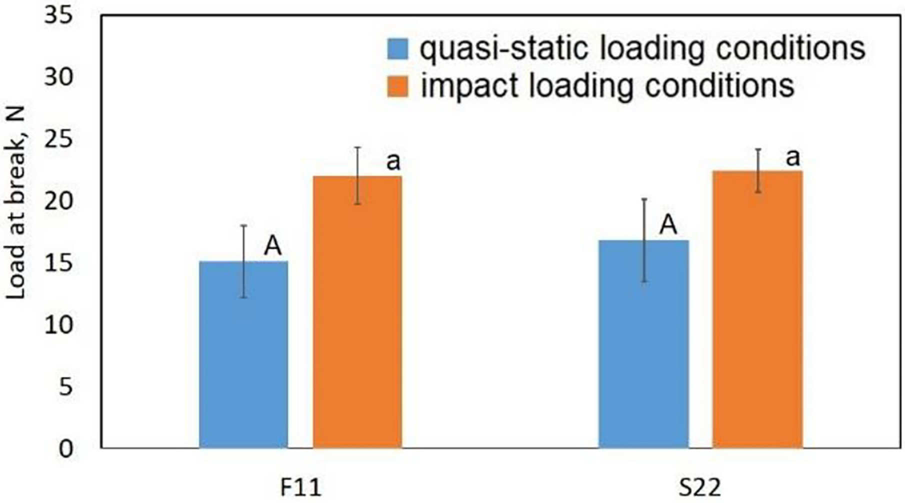
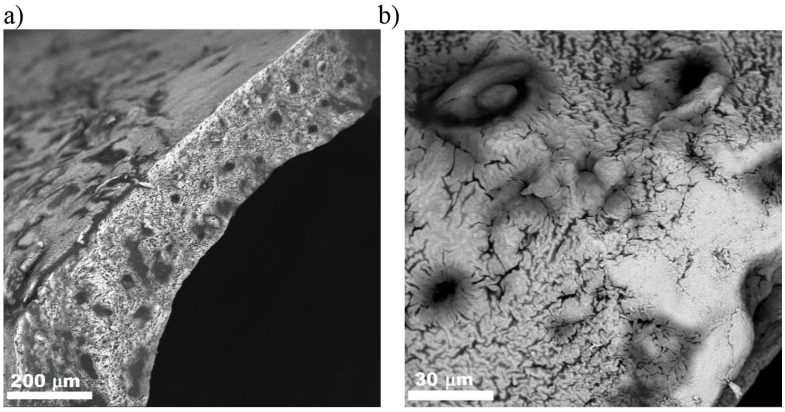
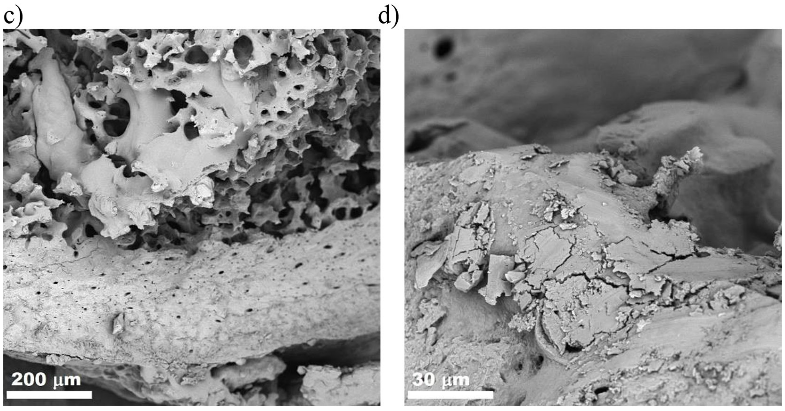


Figure 6. Mean values of load at break with standard deviations for eggs of F11 and S22 quail lines under quasi-static (blue bar) and impact (orange bar) loading conditions. The letters 'A' and 'a' means no statistically significant differences in eggshell strength between tested quail lines under quasi-static and impact loading conditions, respectively.

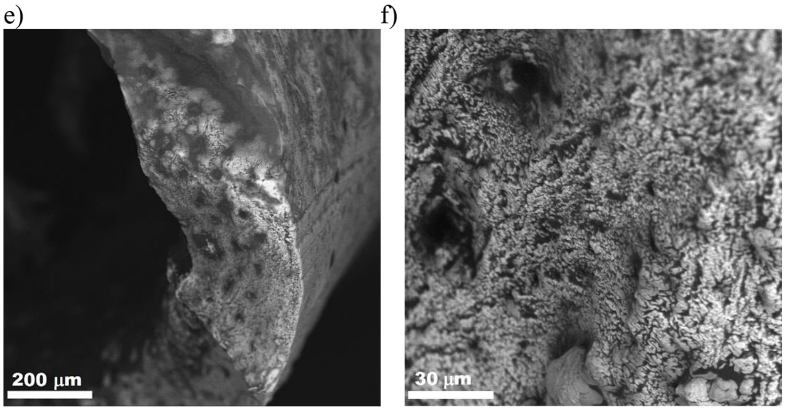
F11 male



F11 female



S22 male



S22 female

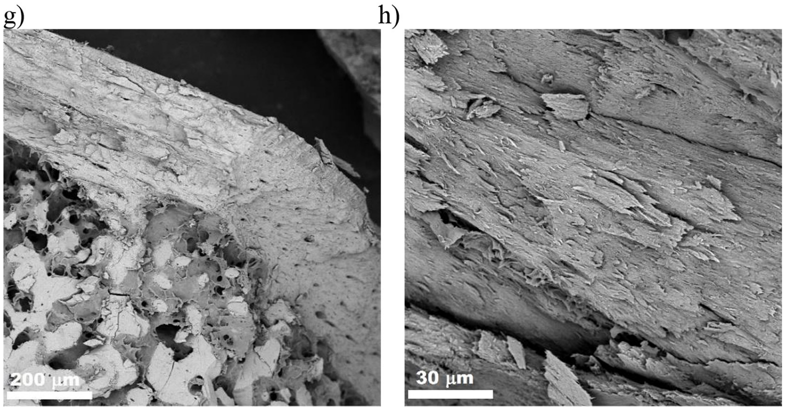


Figure 7. SEM images of femurs fracture surfaces (a, b – F11 male; c, d – F11 female; e, f – S22 male; g, h – S22 female)

Table 2. SEM–EDS elemental analysis. Given mean values are expressed in percent by weight with standard deviations. Statistically significant differences between groups (Tukey´s tests) are indicated by a, b, c

Parameters		O %	Ca %	P %	N %	Na %	Mg %	K %	Ca/P
F11	Male	57.61 ± 7.25^{a}	$19.77 \pm 4.53^{a,b}$	13.22 ± 2.76^{a}	7.67 ± 1.90^{a}	0.94 ± 0.19^{a}	$0.45 \pm 0.1\,2^{a}$	0.34 ± 0.02^{a}	1.50 ± 0.20^{a}
	Female	42.67 ± 1.04^{a}	30.73 ± 2.58^{b}	12.46 ± 0.60^{a}	12.52 ± 0.68^{a}	0.75 ± 0.16^{a}	0.43 ± 0.15^{a}	0.46 ± 0.06^{a}	2.47 ± 0.33^{b}
S22	Male	64.22 ± 7.91^{a}	13.60 ± 2.88^{a}	10.23 ± 3.10^{a}	10.07 ± 2.81^{a}	1.02 ± 0.49^{a}	0.36 ± 0.13^{a}	0.54 ± 0.12^{a}	1.33 ± 0.13^{a}
	Female	52.87 ± 3.41^{a}	$24.67 \pm 1.67^{a,b}$	13.03 ± 1.74^{a}	7.76 ± 0.04^{a}	0.87 ± 0.03^{a}	0.61 ± 0.11^{a}	0.19 ± 0.02^{a}	$1.89 \pm 0.13^{a,b}$

work-to-fracture between analyzed quail lines. They noticed that among the analyzed lines, the Pharaoh breed was characterized by the greatest stiffness. In our study, this quail breed also had the greatest value of most analyzed mechanical parameters. F11 as a meat breed was characterized by greater mass than S22 quails. F11 femurs were also slightly longer. Obtained stiffness values were greater for females 162.27 ± 13.93 and 139.26 ± 22.32 $N \cdot mm^{-2}$ than for males 156.08 ± 14.76 and 135.88 ± 14.72 $N \cdot mm^{-2}$ for F11 and S22 lines respectively. Stiffness depends on bone geometry and bone material properties [20]. Therefore F11 femurs, as longer, could be stiffer. According to Kaczanowska-Taraszkiewicz [6] quail females could have better mechanical properties than males due to the medullary bone presence. In our study birds were 24 weeks old, at the end of their reproductive utility. Therefore probably due to osteoporosis, the bones of females were weaker than those of males as indicated by the results of the impact test (Table 2). Our previous study [21] showed that the impact test was more sensitive than quasi-static three-point bending and allowed for differentiation of the examined rat bone groups. In this research, we also observed that only under impact loading conditions we were able to obtain significantly different values of all analyzed parameters for females and males within a given quail breed.

The main bone component is hydroxyapatite $Ca_5(PO_4)_3(OH)$. The calcium to phosphorus ratio (Ca/P) reflects the mineralization degree and the healthy bone status [22]. The Ca/P molar ratio is < 1.67 in the biological hydroxyapatite. The ratio depends on sex, age, and bone type, and osteoporotic bone has a smaller value than that of healthy bone [23]. Literature reports indicate that osteoporotic bones may also have a higher calcium and lower phosphorus content than healthy bones [24]. In our paper, a higher Ca content and simultaneously higher Ca/P ratio were observed for the female femurs. It could be related to the process of medullary bone formation during the laying period. For laying hens medullary bone content increases with age while structural bone integrity and fracture resistance decrease as impact tests demonstrated. The presence of nitrogen in EDS results (Table 2) may come from collagen fibrils whereas the oxygen content is related to the presence of organic and inorganic compounds [26, 27]. The obtained values of percentage content are similar to those obtained by other authors using SEM-EDS measurements [28–30].

Eggshell strength is one of the most important egg quality parameters. It reflects the mechanical and physical properties of the egg and depends on its thickness, mass, morphology, structure, and chemical composition [25]. The eggshell is made of calcium carbonate and contains proteins interacting with the mineral phase controlling its formation and structural organization, thus determining the mechanical properties [31]. A characteristic trait of Japanese quail eggs is the spotted pattern on the shell which is an individual feature for each female [32, 33]. The eggshell color also affects its quality characteristics. For example, studies described by Drabik et al. [32] showed that brown-shelled eggs were characterized by a more resistant shell than blue ones. In this manuscript, a new approach was used to determine quail eggshell strength in which impact loading conditions were applied. Analyzed eggs had similar traits and shell color. Values of forces needed to break the eggshell continuity recorded under impact loading conditions were higher than those obtained in the quasi-static compression test. Our results are in agreement with data obtained by Trnka et al. [11] who studied goose eggs behavior under dynamic loading conditions and found that eggshell strength at high loading rates was higher than that obtained under quasi-static loading.

CONCLUSIONS

The conducted research showed that quail bone strength and eggshell strength depend on the applied mechanical load. The following conclusions can be drawn:

1. The impact test, being more sensitive, can differentiate bones based on their mechanical properties Under quasi-static loading conditions, no significant differences were observed in the strength, stress, work-to-fracture, and modulus of elasticity between male and female femurs. However, significant differences in the values of the analyzed mechanical parameters (strength, stress, work-to-fracture) were obtained under impact loading conditions.
2. The eggshell strength measured under impact loading conditions had greater values than those under quasi-static conditions. Performed analysis of variance showed no significant differences between analyzed quail lines measured under both mechanical load conditions.
3. SEM-EDS qualitative elemental distribution analysis showed a higher calcium content in the female femurs. The damage visible on the fracture surface indicates that these bones were more brittle.

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REFERENCES

1. Whitehead C.C. Overview of bone biology in the egg-laying hen. Poultry Sci. 2004; 83: 193–199.
2. Dumont E.R. Bone density and the lightweight skeletons of birds, Proc. R. Soc. B 2010; 277: 2193–2198.
3. Tykałowski B., Stenzel T., Koncicki A. Selected problems related to ossification processes and their disorders in birds. Medycyna Wet. 2010; 66(7): 464–469.
4. Rodriguez-Navarro A.B., McCormack H.M., Fleming R.H., Alvarez-Lloret P., Romero-Pastor J., Dominguez-Gasca N., Prozorov T., Dunn I.C. Influence of physical activity on tibial bone material properties in laying hens, J Struct Biol. 2018; 201(1): 36–45.
5. Olgun O. The effect of dietary cadmium supplementation on performance, egg quality, tibia biomechanical properties and eggshell and bone mineralisation in laying quails, Animals 2015; 9(8): 1298–1303.
6. Kaczanowska-Taraszkiewicz E. The effect of age and sex on some developmental parameters of quail (Coturnix coturnix Pharaoh) skeletal system. Medycyna Wet. 2001; 57: 510–514.
7. Andrade K., Cruz F., Kaneko I., Nascimento M., Iwaki L., Santos T. Daily egg-cycle in Japanese Quail: serum biochemistry, bones, and oviduct changes. Braz. J. Poult. Sci. 2023; 25(2), eRBCA-2021-1599.
8. Holmes D.J., Ottinger M.A. Birds as long-lived animal models for the study of aging. Exp. Gerontol. 2003; 38: 1365–1375.
9. Suzer B., Petek M., Tufekci K., Arican I., Abdourhamane I.M., Yildiz H. Comparison of some biomechanical properties of tibiotarsus in four different feather color lines of 60-day old female quails. Braz. J. Poult. Sci. 2020; 22(2).
10. Rath N.C., Huff G.R., Huff W.E., Balog J.M. Factors regulating bone maturity and strength in poultry. Poultry Sci. 2000; 79: 1024–1032.
11. Trnka J., Nedomová Š., Kumbár V., Šustr M., Buchar J. A new approach to analyze the dynamic strength of eggs. J Biol Phys. 2016; 42(4): 525–537.
12. Buchar J., Nedomova S., Trnka J., Strnkova J. Behaviour of Japanese quail eggs under mechanical compression, Int. J. Food Prop. 2015; 18: 1110–1118.
13. Nedomova S., Kumbar V., Trnka J., Buchar J. Effect of the loading rate on compressive properties of goose eggs. J. Biol. Phys. 2016; 42(2): 223–233.
14. Macek W., Branco R., Szala M., Marciniak Z., Ulewicz R., Sczygiol N., Kardasz P. Profile and areal surface parameters for fatigue fracture characterisation. Materials 2020; 13: 3691.
15. Nedomova S., Trnka J., Dvorakova P., Buchar J. Hen's eggshell strength under impact loading. J. Food Eng. 2009; 94(3–4): 350–357.
16. Bonagurio L.P., Cruz F.K., Kaneko I.N., Matumoto-Pintro P.T., Murakami A.E., Santos T.C. Dietary supplementation with canthaxanthin and 25-hydroxycholecalciferol has beneficial effects on bone and oxidative metabolism in European quail breeders, Poultry Sci., 2020; 99(10): 4874–4883.
17. Lee J., Tompkins Y., Kim D.H., Kim W.K., Lee K. The effects of myostatin mutation on the tibia bone quality in female Japanese quail before and after sexual maturation, Poultry Sci. 2023; 102(7).
18. Süzer B. Biomechanical comparison of the effects of the storage temperature on tibiotarsus in Japanese quail. J Res Vet Med. 2021; 40(2): 131–135.
19. Skic A., Kołodziej P., Puzio I., Tymicki G., Beer-Lech K., Gołacki K. Methodological aspects of rat bone mechanical impact. Adv Sci Technol-Res. 2022; 16(6): 140–146.
20. Jepsen K.J., Silva M.J., Vashishth D., Guo X.E., van der Meulen M.C. Establishing biomechanical

mechanisms in mouse models: practical guidelines for systematically evaluating phenotypic changes in the diaphyses of long bones. J Bone Miner Res. 2015; 30(6): 951–66.

21. Skic A., Puzio I., Tymicki G., Kołodziej P., Pawłowska-Olszewska M., Skic K., Beer-Lech K., Bieńko M., Gołacki K. Effect of Nesfatin-1 on rat humerus mechanical properties under quasi-static and impact loading conditions. Materials, 2022; 15: 333
22. Kato Y., Yakumaru H., Ayama K., Oikawa M., Homma-Takeda S. Quantitative elemental analysis of rat bone using micro-PIXE analysis. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 2024; 555: 165473.
23. Kourkoumelis N., Balatsoukas I., Tzaphlidou M. Ca/P concentration ratio at different sites of normal and osteoporotic rabbit bones evaluated by Auger and energy dispersive X-ray spectroscopy J. Biol. Phys., 2012; 38: 279–291,
24. Zoehrer R., Perilli E., Kuliwaba J.S., Shapter J.G., Fazzalari N.L., Voelcker N.H. Human bone material characterization: integrated imaging surface investigation of male fragility fractures Osteoporos Int., 2012; 23: 1297–1309.
25. Hassan M.H.A., Geasa M.M.M. Determination of mechanical behavior and physical properties of quail eggs. J. Soil Sci. Agric. Eng, 2022; 13(2): 59–63.
26. Von Euw S., Wang Y., Laurent G., Drouet Ch., Babonneau F., Nassif N., Azaïs T. Bone mineral: new insights into its chemical composition. Sci Rep., 2019; 9: 8456.
27. Roohani I., Cheong S., Wang A. How to build a bone? - Hydroxyapatite or Posner's clusters as bone minerals, Open Ceram., 2021, 6: 100092.
28. Mehta R., Chowdhury P., Ali N. Scanning electron microscope studies of bone samples: Influence of simulated microgravity, Nucl. Instrum. Methods Phys. Res., B, 2007; 261(1–2): 908–912.
29. Prati C., Zamparini F., Botticelli D., Ferri M., Yonezawa D., Piattelli A., Gandolfi M.G. The use of ESEM-EDX as an innovative tool to analyze the mineral structure of peri-implant human bone. Materials 2020; 13: 1671.
30. Imai H., Prati C., Zamparini F., Iezzi G., Botticelli D., Gandolfi M.G., Baba S. ESEM-EDX mineralization and morphological analysis of human retrieved maxillary sinus bone graft biopsies before loading. J. Funct. Biomater. 2023; 14: 391.
31. Gautron J., Stapane L., Le Roy N., Nys Y., Rodriguez-Navarro A.B., Hincke M.T. Avian eggshell biomineralization: an update on its structure, mineralogy and protein tool kit. BMC Mol and Cell Biol, 2021; 22: 11.
32. Drabik K., Batkowska J., Vasiukov K., Pluta A. The impact of eggshell colour on the quality of table and hatching eggs derived from Japanese Quail. Animals, 2020; 10: 264.
33. Sezer M., Tekelioglu O. Quantification of Japanese quail eggshell colour by image analysis. Biol. Res. 2009; 42: 99–105.