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Investigation of the Protective Feature of the Shell Part of Japanese Quail *(Coturnix Coturnix Japonica)* Eggs Against Ionizing Radiation

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ABSTRACT

In this study, we aimed to assess the protective capacity of the eggshell against radiation and the impact of ionizing radiation on the embryonic development process in quail eggs exposed to varying doses of radiation during incubation. A total of 740 quail eggs were divided into six groups, each exposed to different doses of ionizing radiation. Throughout the incubation period, we monitored hatching rates and examined hatched chicks for potential pathologies. Additionally, over six months, we conducted routine weekly examinations and observed the sexual activity of mature quails. At the study's conclusion, clinical pathology was not observed, and there were no mass mortalities. Quails whose laying activities were monitored exhibited normal sexual behavior. Statistical analysis revealed significant differences in hatching rates among the main study groups ($p<0.001$). Specifically, Group VI, exposed to the highest levels of X-rays, exhibited significantly lower hatching rates compared to Groups I and V ($p<0.001$). These findings suggest that the quail eggshell provides some protection against ionizing radiation, as evidenced by the absence of anomalies and the high hatching rates observed in the chicks.

INTRODUCTION

Imaging methods such as direct radiography, fluoroscopy, and computed tomography, commonly utilized for diagnostic purposes in both human and veterinary medicine, represent the primary sources of ionizing radiation exposure (Adalı and Adalı, 2008). Ionizing radiation is well-established to induce cell death or cytogenetic effects through either direct ionization or the indirect interaction of free radicals with crucial cellular components such as DNA (Mayr et al., 1998). The principal cellular mechanisms underlying these effects include fetal cell death, mitotic delay, and disruptions in cell migration (Brent RL, 1989; Mayr et al., 1998). The biological consequences of radiation on fetal tissues typically hinge upon the stage of pregnancy and the radiation dose administered (Mayr et al., 1998; Valentin, 2003; Adalı and Adalı, 2008; Bıçakçı BC, 2009; Shaw et al., 2011). Consequently, exposure to radiation during pregnancy can lead to a spectrum of effects encompassing lethality, teratogenicity, carcinogenicity, genetic mutations, as well as structural and functional abnormalities in the fetus (Mayr et al., 1998; Adalı and Adalı, 2008).

It is widely acknowledged that the most effective means of safeguarding against the deleterious effects of radiation is through the use of protective materials. Currently, lead stands as the predominant choice for such shielding; however, its high cost and density impose limitations on its widespread utilization (Singh et al., 2015; Sevinç and Durgun, 2021). Consequently, alternative materials suitable for radiation protection have been the focus of many studies (Singh et al., 2015; Cherkashina et al., 2020). Exploring unconventional avenues, researchers have investigated the permeability of chicken eggshells, typically considered waste material, to ionizing radiation, with the aim of ascertaining its efficacy as a component in radiation shielding. Several studies have addressed this topic, reporting the eggshell's potential protective properties against radiation (Binici et al., 2013; Binici et al., 2015; Jasmine et al., 2020; Sevinç and Durgun, 2021; Azman et al., 2022). However, a notable gap exists in the literature regarding scientific investigations evaluating the protective capabilities of both chicken and quail eggshells during embryonic development (incubation).

In this study, inspired by previous research suggesting the protective potential of chicken eggshells against radiation, quail eggs were exposed to radiation during incubation to assess both the protective properties of the eggshell and the effects of ionizing radiation on hatching and subsequent embryonic development.

MATERIALS AND METHODS

Animal Material and Creation of Groups

This study, approved by Siirt University Animal Experiments Local Ethics Committee (HADYEK) under decision number 2016/20 and conducted at Siirt University Experimental Animals Application and Research Center (DEHAM), utilized 740 Japanese quail (*Coturnix Coturnix Japonica*) eggs sourced from Siirt University-Wildlife Center, which were divided into 6 main groups and placed in a T series Incubator (Çimuka, Ankara) to initiate the incubation process under controlled conditions (37.7°C temperature and 50-55% humidity). X-ray exposure, according to the predetermined groups, was carried out using a portable X-ray machine (FPX-F3200 portable X-ray, Fuji) at a film-focus distance of 90 cm, with settings of 50 kV and 2.5 mAs dose, with a maximum duration of 3 minutes for egg removal and reinsertion into the incubator for radiography. The X-rays were applied to the eggs within the same group from the same direction simultaneously.

Formation of Working Groups and Group-Based Radiological Applications

Group I (n=100): Served as the control group, with eggs not exposed to X-rays.
Group II (n=240): Divided into 15 subgroups with 16 eggs each, undergoing one radiographic exposure on a matching study day.
Group III (n=100): Received a total of 5 radiographs, initially on the first study day and subsequently every 3 days.
Group IV (n=100): Subjected to 15 daily radiographic imaging procedures.
Group V (n=100): Underwent radiographic imaging 5 times daily (75 images over 15 days), consecutively.
Group VI (n=100): Exposed to radiographic imaging 10 times daily (150 shots over 15 days), every other day.

Following the incubation period, hatched chicks were transferred to cages illuminated with white fluorescent light at a temperature of 35°C and humidity of 30%. Cage conditions were adjusted to maintain a temperature of 30°C during the second week. Post-hatching, chicks were provided with 5% sugar water every 4-6 hours until reaching 3 weeks of age, subsequently transitioning to ground broiler chick feed with ad libitum access to water for the duration of the study. At three weeks of age, quails were relocated to laying cages according to their respective study groups, irrespective of gender, and continued to be fed broiler chick feed until reaching 6 months of age. Throughout this 6-month period, all chicks underwent systematic weekly macroscopic examinations, evaluating for visual impairments, skin lesions, hair structure anomalies, as well as skull and limb abnormalities.

Statistical analysis

The variables in the study were quantified using both absolute numbers (n) and percentages (%). Statistical comparisons between groups were conducted using Fisher-Freeman-Halton and Pearson chi-square tests. Data analysis was performed using the IBM SPSS Statistics 20 software package. Statistical significance was defined as p<0.001.

RESULTS

Examination Findings

Throughout the 6-month period following their transfer from the hatching unit, weekly examinations of the chicks revealed no instances of clinical pathology. Additionally, no mass mortality events were observed during this timeframe. Quails, whose laying activities were monitored, exhibited normal sexual behavior throughout the study duration.

Statistical Findings

The data regarding the number of eggs, number of chicks hatched, and corresponding hatching rates for each group are outlined in Table 1.

Table 1. The number of eggs incubated, number of chicks hatched, and hatching rates by groups

Group	Number of Incubated Eggs (n)	Number of Hatches	Hatching Rate (%)
I	100	99	99[a, b, c, d, e]
II	240	225	95[a, f, g, h, i]
III	100	99	99[b, f, j, k, l]
IV	100	98	98[c, g, j, m, n]
V	100	94	94[d, h, k, m, o]
VI	100	83	83[e, i, l, n, o]

a: Significance of association (X^2) between Group I and II in terms of hatching rates (P= 0.037).
b: Significance of association (X2) between Group I and III in terms of hatching rates (P= 1).
c: Significance of association (X^2) between Group I and IV in terms of hatching rates (P= 1).
d: Significance of association (X^2) between Group I and V in terms of hatching rates (P=0.54).
e: Significance of association (X^2) between Group I and VI in terms of hatching rates (P<0.001).
f: Significance of association (X^2) between Group II and III in terms of hatching rates (P=0.037).
g: Significance of association (X^2) between Group II and IV in terms of hatching rates (P=0.010).
h: Significance of association (X^2) between Group II and V in terms of hatching rates (P=0.93).
i: Significance of association (X^2) between Group II and VI in terms of hatching rates (P=0.001).
j: Significance of association (X^2) between Group III and IV in terms of hatching rates (P=0.56).

k: Significance of association (X^2) between Group III and V in terms of hatching rates (P=0.54).
l: Significance of association (X^2) between Group III and VI in terms of hatching rates (P<0.001).
m: Significance of association (X^2) between Group IV and V in terms of hatching rates (P=0.14).
n: Significance of association (X^2) between Group IV and VI in terms of hatching rates (P<0.001).
O: Significance of association (X^2) between Group V and VI in terms of hatching rates (P=0.014).

The differences in hatching rates among the main study groups were statistically significant (p<0.001). Specifically, the hatching rate of eggs in Group VI, which received the highest X-ray exposure, was significantly lower (p<0.001) compared to both Group I, Group III and Group IV. Notably, there was no statistical difference in hatching rates between Group V, exposed to the second-highest X-ray dose after Group VI, and Group I. Furthermore, the hatching rate of Group I exceeded that of Group II (p<0.001), while no significant difference (p<0.001) was observed among the 15 subgroups within Group II regarding hatching rates.

DISCUSSION AND CONCLUSION

In recent years, the proliferation of radiation-related applications, particularly in fields such as medicine, the energy industry, and the military, has surged. In tandem with these advancements, research into radiation protection methods has become increasingly imperative (Binici et al., 2013; Sevinç and Durgun, 2021). Presently, available data on the subject primarily stem from two sources: experimental data derived from laboratory animals with short gestation periods and observational data gleaned from humans inadvertently exposed to radiation or survivors of nuclear incidents (Mayr et al., 1998; Shaw et al., 2011). When reviewing the existing literature, it becomes evident that studies have predominantly centered around the potential applications of various materials for radiation protection, particularly as building materials (More et al., 2021). Eggshells have emerged as one such material of interest, with investigations into their effectiveness. However, scientific inquiry into the protective capabilities of eggshells against ionizing radiation exposure during embryonic or fetal development is notably lacking. The present study endeavors to fill this gap by assessing the protective properties of quail eggshells against ionizing radiation, with a particular focus on hatchability rates and the observation of radiation-related pathologies post-hatching.

In this study, no macroscopic pathology was detected in the chicks during weekly examinations, and normal laying activities were observed in the quails that reached adulthood. It is well-established that ionizing radiation can directly impact fetal development, with the sensitivity of rapidly dividing and mitotic phase cells being particularly noteworthy. Consequently, the effects of radiation exposure on organisms may manifest as prenatal or postnatal mortality, congenital anomalies, growth disorders, and various structural and functional impairments (Mayr et al., 1998; Bıçakçı, 2009). Considering the absence of any pathology in chicks exposed to ionizing radiation in our study, two interpretations can be proposed. Firstly, it's possible that the eggshell offers comprehensive protection against radiation. Alternatively, it's plausible that the cumulative radiation dose necessary to induce anomaly formation during the embryonal process was not reached. Further research is warranted to elucidate the precise mechanisms underlying these observations and to ascertain the extent of eggshell protection against radiation during embryonic development.

In the present study, significant differences in hatching rates were observed among quail eggs exposed to different doses of ionizing radiation (Table 1). This finding suggests that hatching rates may indeed vary depending on the level of radiation exposure. Consequently, it is reasonable to infer that the eggshell provides protection against radiation to varying degrees, as evidenced by the differential hatching rates observed across the study groups. The observation that the hatching rate of eggs from Group VI, which received the highest radiation exposure, was lower than that of Group I, the control group (p<0.001), initially suggested an expected proportional decrease in hatching rates with increasing radiation exposure. However, the absence of a statistical difference (p<0.001) in the hatching rate between eggs from Group V, which received the second-highest radiation dose after Group VI, and eggs from Group I, challenges this expectation. The disparity in hatch rates between Group V and Group VI, both subjected to daily ionizing radiation throughout the study, underscores the significance of radiation dose as the sole variable distinguishing these groups, as well as the efficacy of the eggshell's permeability or protection against this dose. Existing literature on this topic is limited, as observed in previous studies (Binici et al., 2013; Binici et al., 2015; Jasmine et al., 2020; Sevinç and Durgun, 2021; Azman et al., 2022), which primarily focus on testing various materials for their radiation permeability as potential building materials. Indeed, previous scientific studies have reported that eggshells, including those from chicken eggs, possess the capability to absorb radiation, thereby exhibiting protective properties against it (Binici et al., 2013; Bani-Ahmad et al., 2022). Binici et al., (2013) determined that eggshell and waste battery charcoal could be utilized as radiation-shielding materials in composite materials produced using waste battery charcoal, barite, limestone, cement, sawdust, epoxy, and eggshells. Azman et al., (2022) proposed that bentonite clay blocks, enriched with bentonite clay and eggshell powder (ESP) and reinforced with Bi2O3, WO3, Bi2O3 + GO, or WO3 + GO layers, could serve as an effective radiation shielding material. Sevinç and Durgun, (2021) demonstrated that waste eggshells could be used as an additive to protect against gamma radiation, using composite materials made from waste sawdust, waste PVC sawdust, waste eggshells, and vermiculite. While the aforementioned studies predominantly focused on chicken eggshells, quail eggs were employed in the present study. The eggs were exposed to radiation throughout the incubation process, particularly during the embryonic stage. Despite the difference in study design, the consistent finding that eggshells provide radiation protection aligns with the existing literatüre.

In conclusion, the high hatch rate observed in quail eggs exposed to radiation, along with the absence of any abnormalities in the hatched chicks, provides evidence supporting the notion that quail eggshells may offer some degree of protection against ionizing radiation. Nevertheless, the inability to measure the radiation dose applied to the quail eggs using specialized dosimeters is acknowledged as a limiting factor of this study. The present study serves as a preliminary foundation for future investigations on this topic, and it is recommended that subsequent research incorporate standardized radiation measurements in order to conclusively establish the protective properties of quail eggshells against radiation exposure.

Ethical Declaration

This study, approved by Siirt University Animal Experiments Local Ethics Committee (HADYEK) under decision number 2016/20

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Study and data collection: A.G., M.B.A., Analysis and interpretation: N.Ş., M.B.A., Statistical analysis: D.Ö., Literature search and writing: K.S., N.Ş.

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