Correlation among Egg Weight, Hatchability, Hatch Weight, and Egg Shell Bacterial Load of Indigenous Duck in Bangladesh

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Abstract

An experiment was conducted using 290 hatching eggs from scavenging deshi ducks to examine the correlation between egg weight, hatch weight, hatchability, and eggshell bacterial load. The eggs were divided into three groups based on weight: G1 (<55 g), G2 (55-60 g), and G3 (>60 g), each with three replications. Bacteriological analysis was performed to assess microbial load. Results showed significant differences ($p<0.01$, $p<0.05$) in egg weights among the groups. Hatch weight also varied significantly ($p<0.05$) between G1 and the heavier groups (G2 and G3). A strong positive relationship was observed between egg weight and chick hatch weight, with G3 producing the heaviest chicks (38.5 g) from the largest eggs (63.45 g). The correlation between egg weight and hatch weight was significant ($p<0.05$, $r = 0.633$), and a very strong positive relationship was found between egg weight and hatchability ($p<0.05$, $r = 0.99$). Fertility and hatchability also showed a strong positive correlation ($r = 0.70$). Microbial analysis revealed that E. coli was more prevalent than Salmonella sp. (27.77% vs. 18.88%), with both bacteria more common on the eggshell surface than in the inner contents. An irregular positive relationship was noted between microbial load (E. coli and Salmonella sp.) and hatchability. The study concluded that larger egg size (>60 g) at the time of setting positively influences fertility, hatchability, and chick hatch weight. However, the fertility and hatchability rates of scavenging duck eggs were below standard levels, highlighting the need to educate rural farmers on improving these parameters for better productivity. Overall, the findings emphasize the advantages of selecting larger eggs for hatching and addressing microbial contamination to enhance hatchability outcomes.

Keywords: Correlation, Fertility, Hatchability, Egg weight, Hatch weight, Duck

Significance | This study determined no significant link between serum Vitamin D levels and COVID-19 mortality, except with LDH.

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1. Introduction

Duck, among other poultry species, is considered an economic livelihood option in Bangladesh, especially for smallholders and women who raise ducks in conjunction with other agricultural operations, where usually no extra feed is required. The duck population has been estimated at 68.26 million, occupying the second position in poultry in Bangladesh (DLS, 2024). Bangladesh has the third-largest duck population after China and Indonesia (Dolberg, 2008). Ducks play a vital role in income generation, nutritional fulfillment, and employment generation in low-lying areas of Bangladesh (Islam et al., 2003). People rear ducks with low inputs and obtain quick returns in terms of eggs and meat, which are usually used for domestic consumption and as a source of good

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and quick earnings. It is an important component of the farming system and plays a significant role in 80% of the rural population in Bangladesh (Islam et al., 2012). The majority of ducks are reared by smallholders in coastal and low-lying areas under a scavenging system, with little or no supplementation (Rahman et al., 2020). Ducks eat slugs and weeds, and have a less aggressive pecking order than chickens. Therefore, indigenous ducks generate income and provide protein to people. Consequently, they are believed to act as indispensable "change makers" in the rural economy of Bangladesh (Hamid 2019).

Nondescript local ducks are ubiquitous in Bangladesh, and most smallholder farmers keep them under a subsistence level of management (Islam et al., 2003). Duck eggs are quite large compared to chicken eggs (1.4 times), making them easily distinguishable. The duck egg contains relatively less water and a higher percentage of protein and fat in the yolk, albumen, and total contents of the egg compared to chicken eggs. Owing to the higher percentage of fat, its energy value is also higher than that of chicken eggs (Quan et al., 2019). Duck eggs are richer in flavor than chicken eggs, have a better baking quality, and have a longer storage life. People allergic to chicken eggs can consume duck eggs. Duck eggs have higher levels of vitamins and minerals, with 12-13 nutrients, than chicken eggs. Duck eggs are less prone to cracking than chicken eggs because the shell membrane is very strong, which prevents leakage even if the shell is broken. In addition, ducks may have nicer backyard occupants (Danilo et al., 2024). Fertility and hatchability are important economic aspects that signify the major components of reproductive performance, and are sensitive to environmental and genetic factors (Widiyaningrum et al. 2016). Fertility is known as the percentage of embryonated eggs after three days of placement in the incubator; on the other hand, hatchability is the percentage of fertile eggs that hatch (King'ori, 2011; Taplah et al., 2018). Fertility and hatchability are affected by both genetic and non-genetic factors. Among them, non-genetic factors have a larger effect on fertility and hatchability in production (King'ori, 2011), including breeder management, egg quality, and incubation procedures. The management of breeding stock includes genetic selection (Drouilhet et al., 2014), age of the breeders (King'ori, 2011), season and feeding (King'ori, 2011), breeding system and technology (Weis et al., 2011), and egg quality (Hester, 2017). It has been reported that temperature, relative humidity, ventilation, and turning of eggs throughout incubation and hatching are environmental factors that can alter hatchability (Archer et al., 2017; Ramli et al., 2017). External characteristics such as egg size and weight affect hatchability. It has been found that medium-sized eggs from Muscovy ducks have better hatchability than small eggs (Weis et al. 2011). In Bangladesh, eight government hatcheries operate to produce and distribute ducklings to duck producers all over the country, which is far below the demand, especially in haor basins (DLS, 2018). The production of ducklings is influenced by the fertility and hatchability of eggs, which are important economic factors that represent the major components of reproductive performance. Although Bangladesh is rich in duck germplasm, unfortunately, due to its lower productive and reproductive performance, as well as a lack of public and private initiatives to accelerate the development of the duck subsector, it remains suboptimal (Hamid 2019). An insufficient number of hatcheries for quality ducklings in the country is one of the major reasons for the non-accelerated growth of the duck subsector. To date, no studies have investigated the correlation among egg weight, hatchability, hatch weight, and eggshell bacterial load in indigenous ducks.

Therefore, this study aimed to determine the fertility percentage and hatchability of fertile eggs collected from scavenging duck eggs, and the correlation between egg weight, hatchability, and hatch weight, as well as the eggshell bacterial load of indigenous ducks, and their effect on hatchability.

2. Materials and methods

2.1 Collection, selection of hatching eggs, and layout of the experiment

A total of 300 hatching eggs were collected from Indigenous ducks aseptically from retailers in the area of Rajshahi City Corporation, Rajshahi. At each time of collection, precaution was taken to prevent cross-contamination of samples. The eggs were stored at 27^{o} to 28^{o}C (80.6 to 82.4^{o} F) for 2 days. After collection, the eggs were scrutinized on the basis of their physical characteristics: size, shape, weight, shell texture, and cleanliness of the shell. The eggs (270) of good size, uniformly calcified, clean, sound shell (without hairline crack), and standard shape were selected. Then the eggs were divided into three groups based on their weight: G_1 (<55 g), G_2 (55-60 g), and G_3 (>60 g), each with three replicates containing 30 eggs in each replicate (Supplemental **Table 1**). Each of the selected eggs was marked with different symbols for identification of each group.

Figure. 1.

2.2 Egg setting in the incubator, candling, and hatching out of ducklings

The incubator was properly cleaned, disinfected by fumigation before setting the eggs in the incubator. A total of 270 selected hatching eggs were set in the same incubator in 3 groups, having 90 eggs in each, under the same standard temperature, humidity, turning, and ventilation system suggested by the James Way incubator manual. Eggs were automatically turned 3-4 times/d until 21 d of the hatching period (setting period only).

The eggs were candled on the 7^{th} and 14^{th} days of incubation in a dark room held over the electric candler to observe the inside of the eggs to identify the clean or infertile eggs and dead embryos. Infertile eggs were clean with only the shadow of the yolk. No blood

vessels, no ring, no spots in the yolk were detected. These types of eggs were removed from the incubator immediately after detection (**Figure1**).

2.3 *Microbial load analysis of the hatching eggs*

Among the selected indigenous duck eggs, 5 eggs from each replicate sample were prepared as a composite sample (Elafify et al., 2016; El-kholy et al., 2014; Adesiyun et al., 2005) for microbial load determination. Each sample was inserted into sterile zip lock bags with an identification mark. The samples were carried to the laboratory, maintaining the cool chain. The samples were examined as soon as possible for bacteriological analysis. Sterile gloves were worn to handle egg samples from each source. One sterile swab, moistened in sterile saline, was applied to the surface of each egg. The swabs applied to five egg shells were submerged in 6 ml of sterile saline as 'shell wash' and used to inoculate appropriate enrichment broths or media. Different types of media and chemicals were used for this analysis. However, **Figure 2** represents the schematic flow chart of the microbial load assessment.

2.4 Record keeping and data analysis

Selection of suitable hatching eggs was performed after cleaning and disinfection of the collected eggs. Extra-large or very small eggs were discarded from the selection lot. The weight of the individual egg was recorded by a digital balance and marked under the desired group, *i.e.*, G_1 = <50 g, G_2 = 50-60 g, G_3 = >60 g. In this present study, fertility was determined after the 1st and 2nd candling (the 7th and 14th d) of the eggs set in the incubation. The fertility was obtained as a percentage by using the following formula:

$$\text{Fertility\%} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \times 100$$

Further, the hatchability of the experimental eggs was determined on the basis of the total number of fertile eggs that were set into the incubator. It was calculated by using the following formula.

$$\text{Hatchability (\%)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of fertile eggs}} \times 100$$

The data obtained from this study were analyzed by the one-way analysis of variance (ANOVA) technique in a completely randomized design (CRD). Regression and correlation coefficients were determined by the Microsoft Excel program to find out the relationship between the variables. All data were presented as means ± standard deviation (SD). Analysis of variance (ANOVA) was conducted to evaluate the recorded data using Kaleida Graph (Synergy Software, Reading, PA, USA). Fischer's LSD performed comparison tests among the statistically significant differences, and differences were considered statistically significant when the p-value was smaller than 0.05 and 0.01.

3. Results and discussion

3.1 Interrelation between egg weight, hatch weight, fertility, and hatchability

The present study was conducted to find out the correlation between egg weight, hatch weight, fertility, and hatchability. The effects of different treatment groups on egg quality, fertility, hatchability, and hatch weight are represented in **Table 1**.

The results demonstrate that the different treatment groups (G1, G2, and G3) had significant effects on all measured parameters ($p < 0.01$). Egg weight progressively increased from G_1 to G_3. G_3 exhibited the highest egg weight (63.42 g), significantly greater than both G_2 (57.33 g) and G_1 (51.48 g). The distinct superscripts (a, b, c) confirm significant differences between the groups. The difference between the egg weights (g/egg) may be due to differences in duck age, rearing condition, and care of eggs before hatching. Fertility rates varied significantly among the groups. Interestingly, G_3 showed the highest fertility (74.50%), while G_2 recorded the lowest (55.63%). The lower fertility in G_2 compared to G_1 (62.25%) suggests that the conditions or treatments in G_2 were less favorable for optimal fertilization. Hatchability followed a similar trend to fertility, being lowest in G_1 (24.50%) and highest in G_3 (50.25%). The improvement in hatchability from G_1 to G_3 indicates that the treatment applied to G_3 eggs may have enhanced embryonic survival and development. Hatch weight was also significantly affected. Chicks from G_3 had the highest hatch weight (38.5 g), while those from G_1 had the lowest (32.00 g). G_2 (36.4 g) showed intermediate results but shared statistical similarity with both G_1 and G_3 as indicated by the common superscripts (ab).

There is a positive relationship between egg weight and hatch weight; the highest chick weight was found in G_3 (38.5g), which had the highest egg weight (63.45g) (**Table 1**). Farooq et al. (2001) reported similar findings, noting a positive association between egg weight and chick hatch weight. Chick hatch weight has been reported to be 62 to 72% and 68.2 % of the initial egg weight, as reported by Wilson (1991) and Murad et al. (2001), respectively. Heavier eggs contain more nutrients than small eggs (Williams, 1994). As a result, developing embryos from heavier eggs tend to have more nutrients for their growth requirements. Similarly, chicks from heavier eggs tend to have more yolk attachment at hatching (Birkhead and Nettleship, 1982; Wolanski et al.,2006). Chicks tend to depend on this yolk during the first few hours after hatching (Deeming, 1989). The chicks utilize the yolk attachment after hatching, and the potential performance of day-old chicks may depend on the quality and quantity of this yolk. The present findings are similar to those of Gonzalez et al. (1999), who observed that chicks from heavier ostrich eggs had a higher hatch weight than those from lighter eggs. In this research, Group 3 (G_3) eggs, with the largest average weight (63.42 g), showed the highest fertility rate (74.50%), while G_2 eggs (57.33 g) displayed the lowest fertility rate (55.63%). This indicates a favorable link between egg weight and fertility up to a certain threshold. It was also noted that medium-sized eggs had superior fertility rates relative to both smaller and larger eggs, suggesting that excessively large or tiny eggs may experience diminished fertility. Hatchability increased with greater

Table 1. Effect of different treatment groups on egg quality, fertility, hatchability, and hatch weight

Variables	Mean ± SD			p value
	G_1	G_2	G_3	
Egg weight (g)	51.48^{a} ± 2.40	57.33^{b}± 1.53	63.42^{c} ± 2.59	< .0001
Fertility (%)	62.25^{a} ± 0.35	55.63^{b}± 0.88	74.50^{c} ± 0.70	0.00024
Hatchability (%)	24.50^{a} ± 0.70	33.66^{b} ± 0.47	50.25^{c} ± 0.35	< .0001
Hatch weight (g)	32.00^{a} ± 2.00	36.4^{ab} ± 2.50	38.5^{c} ± 2.88	0.01557

Means with different superscript letters differ significantly, and ** = significant at 01 % level (P <0.01), and n = 30.

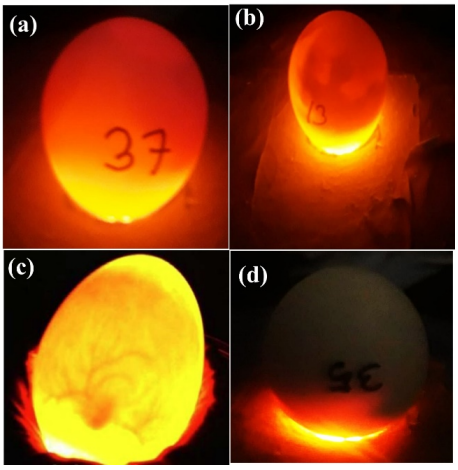


Figure 1. Visualization of the infertile egg (a), fertile egg (b), developing healthy embryo (c), and 14-day growing embryo (d) through candling.

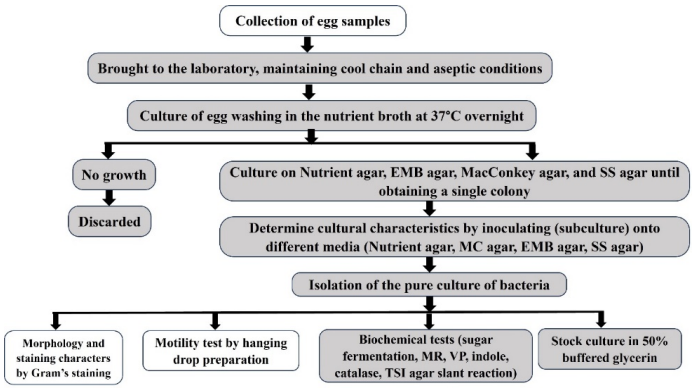


Figure 2. Flow chart of assessing the bacterial load of the duck eggs under various groups.

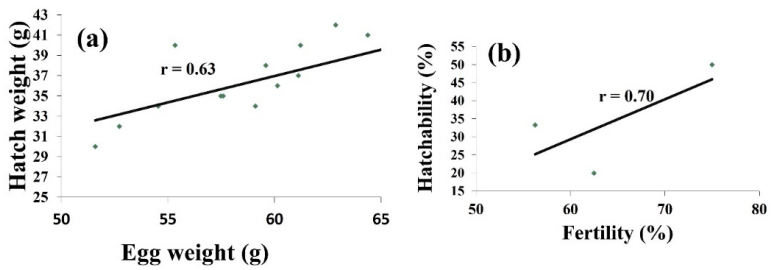


Figure 3. Correlation between egg weight and hatch weight (a) and fertility and hatchability (b).

egg weight, with G_3 exhibiting the greatest hatchability at 50.25% and G_1 the lowest at 24.50%. Ng'ambi et al. (2013) documented a robust positive association (r^2 = 0.727) between egg weight and hatchability in indigenous Venda chickens, reinforcing the premise that larger eggs often exhibit superior hatchability rates.

3.2 Correlation between egg weight and hatch weight, fertility, and hatchability

The correlation between egg weight and hatch weight, as well as fertility and hatchability, is shown in Figure 3 (a), (b), respectively. The correlation coefficient (r) of 0.63 between egg weight and hatch weight indicates a moderate to strong positive relationship. This suggests that, generally, heavier eggs tend to produce heavier chicks at hatching. However, the correlation implies that other factors also influence hatch weight. It's important to note that while egg weight is a significant factor influencing hatch weight, other variables like genetics, incubation conditions, and parental nutrition also play crucial roles in determining chick weight at hatching. Similar findings were observed by Narkhede et al. (1981), who reported a strong and positive correlation (r2 = 0.93) between egg weight and chick hatch-weight in crossbred chickens (Rhode Island Red X White Leghorn). However, Asuquo & Okon (1993) observed that egg size within the intermediate weight range of 45 to 56 g hatched heavier chicks than smaller or larger eggs. No biological reasons were suggested for such an observation. Nahm (2001) stated that there are strong positive correlations between pre-incubation egg weight and chick weight and the subsequent performance of chickens. Farooq et al. (2001) studied that the size of newly hatched chicks is directly related to the size of the hatchling egg, and there is a progressive increase in egg production related to hatching egg weight. The smaller weight of the newly hatched chick is attributable to smaller egg weight (Khurshid et al., 2003).

However, a strong positive correlation between egg weight and hatchling weight, which is constant across species (Wilson, 1991). A study by Pinchasov (1991) reported a high correlation (r = 0.89) between egg weight and hatch weight in broiler chicks, indicating that larger eggs consistently resulted in heavier hatchlings. Chimezie et al. (2024) found a very strong correlation between egg weight and hatch weight ($r \approx 0.97$) in Japanese quail, suggesting that egg weight is a reliable predictor of hatch weight in this species. Furthermore, Ng'ambi et al. (2013) observed a correlation coefficient (r^2 = 0.953) between egg weight and chick hatch weight, further supporting the positive relationship across different poultry breeds. However, these studies report varying strengths of correlation, they collectively reinforce the general trend that heavier eggs are likely to yield heavier chicks at hatching. It is assumed that the differences in correlation coefficients across studies may be attributed to factors such as breed differences, environmental conditions, and management practices.

A correlation coefficient (r) of 0.70 (Fig. 3 b) suggests a strong, positive relationship between fertility and hatchability. The trend line indicates that as fertility increases, hatchability also increases. In simpler terms, it implies flocks with better fertility tend to have better hatchability. A similar positive relationship between fertility and hatchability in indigenous chickens was reported by Ng'ambi et al. (2013), who showed that eggs with higher fertility rates led to improved hatchability rates. It was reported that 71.16 % hatchability based on fertile eggs and poor eggshell weight has also been reported to result in higher embryonic mortality influenced by egg weight (Farooq et al. 2001). It was observed that lower hatchability was observed in a flock if embryonic mortality is higher and fertility is lower (Roque and Soares 1994). Furthermore, Wilson (1991) also highlighted that fertility is a critical factor influencing hatchability outcomes, and lower fertility often leads to a lower number of viable embryos, which directly affects hatchability.

3.3 *Correlation between bacterial load and hatchability*

The overall prevalence of E. coli and Salmonella sp. according to egg weight of duck eggs was analyzed, and found that *E. coli* prevalence was more prominent than *Salmonella spp.* The growth of bacteria in various media is shown in Figure 4. The growth of *E. coli* in nutrient broth was characterized by the development of diffused turbidity (Figure 4a), and growth on nutrient agar (Figure. 4a) was indicated by the development of smooth, circular, white to grayish white colonies. Further, the growth of *E. coli* on EMB (Figure 4c) was indicated by the development of smooth, circular, black color colonies with metallic sheen, and the growth of Salmonella sp. was indicated by the development of colorless colonies with a black center (Figure 4d).

Further, the isolated bacteria were identified by the sugar fermentation test. The fermentative reactions of isolated bacteria with five basic sugars were determined, and isolated *E. coli* fermented dextrose, lactose, sucrose, maltose, and mannitol with the production of acid and gas (Figure 5a). Isolated *Salmonella spp.* also fermented those sugars except lactose and sucrose. The catalase test was performed to differentiate catalase enzyme-producing bacteria from those of not producing it. In this test, isolated E. coli and Salmonella sp. showed a positive result, indicating catalase-producing bacteria (Figure 5 b). Moreover, isolated E. coli showed a positive result for the indole test, which appears as a red color (Fig ure 5c). The methyl red (MR) test was used to differentiate different genera of the Enterobacteriaceae family. All of the isolates of *E. coli* and *Salmonella spp.* were positive in the MR test (Figure 5d).

The correlation between hatchability and bacterial load (*E. coli*) well as *Salmonella spp*., is shown in Figure 6 (a), (b), respectively.

The finding revealed that there is a weak to moderate negative relationship between E. coli and hatchability in duck eggs (r=0.33) (Figure. 6a). This suggests that as *E. coli* contamination increases, hatchability tends to decrease, though the association is not

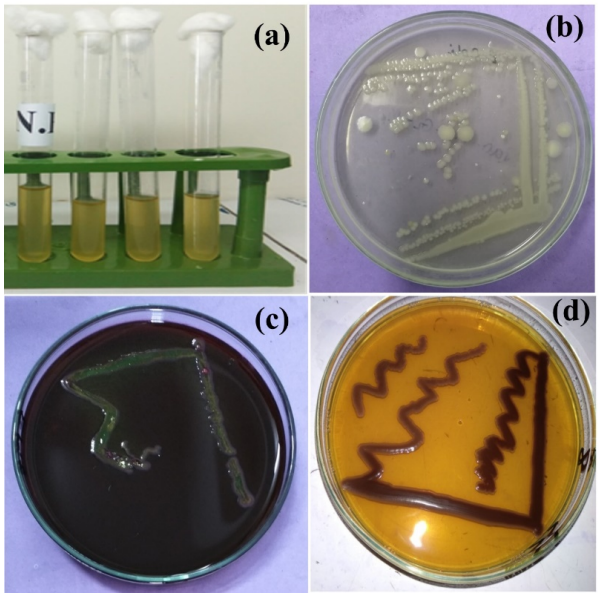


Figure 4. Growth of bacteria in nutrient broth (a), E. Coli on nutrient agar (b), E. Coli on EMB agar (c), and Salmonella on SS agar (d)

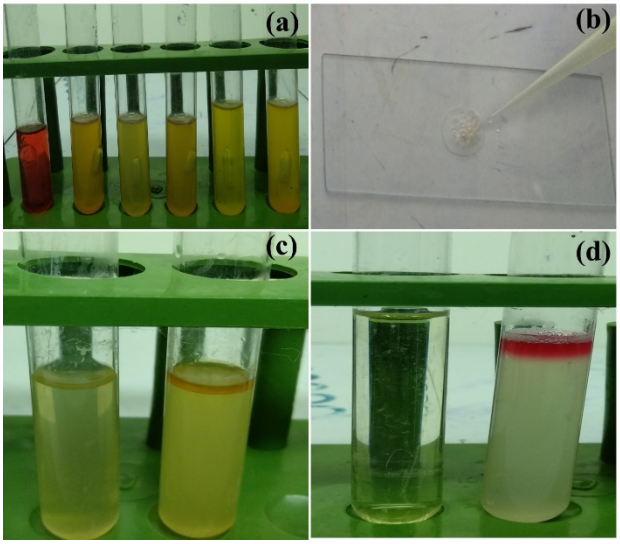


Figure 5. Bacterial confirmation test. Fermentation activity of *E. coli* with five basic sugars (*a*), catalase test (b), indole test (c), and MR test of isolated *E. coli* and *Salmonella spp.* (d).

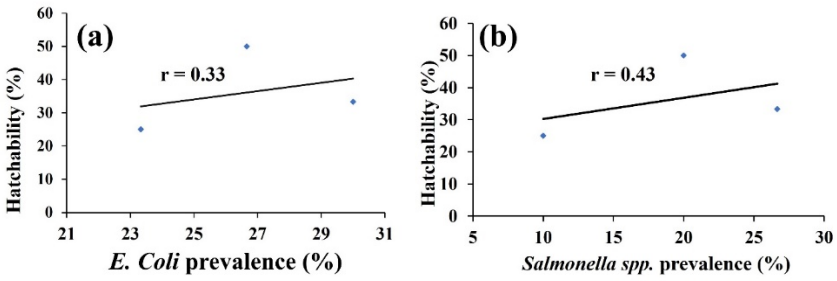


Figure 6. Correlation between bacterial load and hatchability: Here, *E. coli* and hatchability, and *Salmonella spp.* and hatchability are represented by (a) and (b), respectively.

particularly strong. Such a correlation implies that while *E. coli* prevalence adversely affects hatchability; other factors also significantly influence hatch success. A study demonstrated that exposing embryonating eggs to *E. coli* led to increased neonatal chick mortality, highlighting the pathogen's role in reducing hatchability (Karunarathna et al., 2022). A recent study indicated that E. coli infection could result in the death of up to 92% of affected embryos, emphasizing its potential to drastically lower hatchability rates (Oliveira et al., 2024). Furthermore, surveillance studies in Egyptian hatcheries found a 36.3% prevalence of *E. coli* in various samples, including eggs and hatchery equipment, suggesting widespread contamination that could impact hatchability (Yousef et al., 2023).

Furthermore, the relationship between *Salmonella spp*. prevalence in duck eggs and hatchability was analyzed and found that there is an irregular positive relationship between *Salmonella spp*. and hatchability in duck eggs (r=0.43) (Figure 6 b). The correlation coefficient (r) of 0.43 between *Salmonella spp*. prevalence and hatchability indicate a moderate negative relationship. This suggests that as the prevalence of Salmonella increases, hatchability tends to decrease, although the association is not particularly strong. A study demonstrated that exposing embryonating eggs to Salmonella led to increased neonatal chick mortality, highlighting the pathogen's role in reducing hatchability and research in breeder hatcheries found that 11.1% of samples, including egg fragments and fluff, were contaminated with Salmonella, indicating a significant presence that could impact hatchability (Cox et al., 1991). Furthermore, it was reported that infected laying ducks showed reduced egg production and quality, suggesting that *Salmonella* infections can have broader reproductive implications beyond hatchability (Zhang et al., 2019). In another study, found that Salmonella spp. can persist for 4 to 5 years within the hatchery, which causes contamination, and infected chicks may die with peaks in mortality by the 15th day (Chen et al, 2002).

However, the current findings suggest that selecting medium to large-sized eggs for incubation can enhance fertility, hatchability, and chick quality and this study not only sheds light on the biological and microbiological factors influencing duck hatchability but also underscores the broader implications for food safety and economic development in the poultry sector.

Conclusion

The present study successfully demonstrated the significant relationships among egg weight, hatch weight, hatchability, and bacterial contamination of indigenous duck eggs. Heavier eggs were positively correlated with higher hatch weights and better hatchability rates. A positive correlation (r = 0.63) between egg weight and hatch weight emphasizes the biological advantage of setting larger eggs during incubation to optimize hatchling quality. Bacteriological analysis revealed a considerable prevalence of E. coli and Salmonella spp. in duck eggs, with contamination evident in both the eggshell and egg contents. The correlation between bacterial contamination and reduced hatchability (r = 0.33 for E. coli and r = 0.43 for Salmonella spp.) highlights the critical impact of microbial load on reproductive success. Finally, the fertility and hatchability of the scavenging duck eggs are below standard level and therefore, it is very necessary to aware rural farmers for better fertility and hatchability. The results highlight the importance of setting larger eggs for better hatchling quality and maintaining strict hygiene to reduce bacterial contamination. To enhance duck farming profitability and public health safety, coordinated efforts among government, research institutes, NGOs, and industry stakeholders are essential.

Author contributions

M.S.I. and K.M.M.H. selected the title, study objectives, and supervised the research work. F.A.I. conducted the research work and literature review, P.K. edited the references, M.Z.I. wrote the manuscript, and A.H.H. and M.S.I. revised the manuscript. All authors reviewed and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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