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Artificial incubation of ostrich eggs (*Struthio camelus*), on the northeast coast of Brazil

Incubação artificial de ovos de avestruz (*Strutio camelus*) no litoral nordeste do Brasil

Incubación artificial de huevos de avestruz (*Strutio camelus*) en la costa noreste de Brasil

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Abstract
This study aimed to evaluate data collected on ostrich eggs in a commercial hatchery, focusing on physical characteristics, incubation temperature, relative humidity inside the incubator, egg fertility, embryonic mortality, egg mass at collection, mass loss during artificial incubation, chick mass at hatching, shell weight, and hatching rate. The eggs were cleaned with paper towels, individually identified, and weighed at collection and subsequently on days 14, 28, and 39 of incubation. Prior to incubation, the eggs were stored for four days at 18–20 °C and 50–60% relative humidity. Incubation was carried out at 36–37 °C and 25–35% relative humidity. Candling was performed at the beginning of incubation and on days 14, 28, and 39 to assess embryonic development. At hatching, both chicks and eggshells were weighed. Of the 120 eggs, 101 were fertile (84.16%) and 19 (15.83%) infertile; among the fertile eggs, 14 (13.86%) resulted in embryonic mortality. A total of 87 chicks hatched, corresponding to a hatchability rate of 86.13% among fertile eggs. The mean initial egg mass was 1,308.78 ± 157.08 g, and the mean chick mass at hatching was 815.56 ± 129.23 g, representing 61.13% of the initial egg mass. The mean egg mass loss during incubation was 19.97 ± 5.18%, a value higher than those reported in previous studies.
Keywords: Hatchability; Egg mass loss; Embryonic development.

Resumo
Este estudo teve como objetivo avaliar dados coletados sobre ovos de avestruz em uma incubadora comercial, com foco em características físicas, temperatura de incubação, umidade relativa dentro da incubadora, fertilidade dos ovos, mortalidade embrionária, massa dos ovos na coleta, perda de massa durante a incubação artificial, massa dos pintinhos na eclosão, peso da casca e taxa de eclosão. Os ovos foram limpos com papel toalha, identificados individualmente e pesados na coleta e, posteriormente, nos dias 14, 28 e 39 de incubação. Antes da incubação, os ovos foram armazenados por quatro dias a 18–20 °C e 50–60% de umidade relativa. A incubação foi realizada a 36–37 °C e 25–35% de umidade relativa. A ovoscopia foi realizada no início da incubação e nos dias 14, 28 e 39 para avaliar o desenvolvimento embrionário. Na eclosão, tanto os pintinhos quanto as cascas dos ovos foram pesados. Dos 120 ovos, 101 eram férteis (84,16%) e 19 (15,83%) inférteis; entre os ovos férteis, 14 (13,86%) resultaram em mortalidade embrionária. Um total de 87 pintinhos eclodiram, correspondendo a uma taxa de eclosão de 86,13% entre os ovos férteis. A massa média inicial do ovo foi de 1.308,78 ± 157,08 g, e a massa média do pintinho na eclosão foi de 815,56 ± 129,23 g, representando 61,13% da massa inicial do ovo. A perda média de massa do ovo durante a incubação foi de 19,97 ± 5,18%, um valor superior aos relatados em estudos anteriores.
Palavras-chave: Eclosão; Perda de massa do ovo; Desenvolvimento embrionário.

Resumen
Este estudio tuvo como objetivo evaluar los datos recopilados sobre huevos de avestruz en una incubadora comercial, centrándose en las características físicas, la temperatura de incubación, la humedad relativa dentro de la incubadora, la fertilidad de los huevos, la mortalidad embrionaria, la masa del huevo al momento de la recolección, la pérdida de masa durante la incubación artificial, la masa del polluelo al nacer, el peso de la cáscara y la tasa de eclosión. Los huevos se limpiaron con toallas de papel, se identificaron individualmente y se pesaron al momento de la recolección y posteriormente a los 14, 28 y 39 días de incubación. Antes de la incubación, los huevos se almacenaron durante cuatro días a 18-20 °C y 50-60 % de humedad relativa. La incubación se llevó a cabo a 36-37 °C y 25-35 % de humedad relativa. Se realizó ovoscopia al inicio de la incubación y a los 14, 28 y 39 días para evaluar el desarrollo

embrionario. Al nacer, se pesaron tanto los polluelos como las cáscaras de huevo. De los 120 huevos, 101 fueron fértiles (84,16 %) y 19 (15,83 %) infértiles; de los huevos fértiles, 14 (13,86 %) presentaron mortalidad embrionaria. Nacieron 87 polluelos, lo que corresponde a una tasa de eclosión del 86,13 % entre los huevos fértiles. La masa inicial promedio del huevo fue de 1308,78 ± 157,08 g, y la masa promedio del polluelo al nacer fue de 815,56 ± 129,23 g, lo que representa el 61,13 % de la masa inicial del huevo. La pérdida promedio de masa del huevo durante la incubación fue del 19,97 ± 5,18 %, un valor superior a los reportados en estudios previos.
Palabras clave: Eclosión; Pérdida de masa del huevo; Desarrollo embrionario.

1. Introduction

It is well established that all stages of ostrich production are important; however, incubation plays a decisive role in the overall success of this activity. Incubation outcomes directly influence the productivity of ostrich farming enterprises (Gurri, 1995a). One of the main factors affecting production results and profitability is the number of chicks produced per breeding female (Gurri, 1995b). More (1996a) highlights the lack of accurate data regarding ostrich production performance from hatching to four months of age in commercial breeding systems.

One of the factors affecting the number of eggs produced by a female ostrich is the age of the bird. Thus, domestication has resulted in early sexual maturity (Ipek & Sahan, 2004). These authors observed changes in egg production and incubation results influenced by the age of the breeder and the breeding season. The breeding season affected the hatching rate, which was 64.3% in the first season, increased with age, and reached 73.1% in the fifth.

This study aimed to evaluate data collected on ostrich eggs in a commercial hatchery, focusing on physical characteristics, incubation temperature, relative humidity inside the incubator, egg fertility, embryonic mortality, egg mass at collection, mass loss during artificial incubation, chick mass at hatching, shell weight, and hatching rate.

These parameters were analyzed and correlated with fertility, infertility, hatching, and embryonic mortality rates in a coastal region of northeastern Brazil.

2. Methodology

2.1 Location, Birds, and Eggs

An experimental, field research was carried out in a quantitative study (Pereira et al., 2018) using simple descriptive statistics with line and bar graphs, with data classes, with mean and standard deviation values and absolute frequency and relative percentage frequency (Shitsuka et al., 2018) and employing statistical analysis (Bekman & Costa Neto, 2009).

This study was conducted using eggs from breeding ostriches over three years of age, sourced from a commercial ostrich farm located in Caucaia, Ceará, Northeastern Brazil, a coastal region along the Atlantic Ocean (latitude: -3.73454, longitude: -38.6563; 3°44′4″ S, 38°39′23″ W). The climate is tropical sub-humid, with a mean annual rainfall of 1,399.9 mm concentrated between January and June. Mean annual temperatures range from 26 to 28 °C, and the mean relative humidity is 74.5%. The company operates a hatchery specializing in the artificial incubation of ostrich eggs. Eggs were collected from breeding pairs housed in 1,000 m² paddocks (20 × 50 m), with 15 pairs maintained under identical management and feeding conditions. Each bird received 1.5 kg/day of a balanced diet (18% crude protein, 2% calcium) composed of ground soybean and corn, supplemented with chopped green forage (*Pennisetum purpureum*), and water was provided *ad libitum.* Immediately after laying, each egg was collected from the nest using a disposable plastic bag to avoid contact with the operator’s hands. Eggs were cleaned, when necessary, using a dry disposable paper towel. Each egg was marked with the female’s identification code and date of collection, then transported to the hatchery. No disinfection by fumigation or washing was performed at any stage of the process.

2.2 Egg, Chick, and Shell Mass

Egg masses were measured using a digital scale with a precision of 5 g. All eggs were weighed at the beginning of storage, on the first day of artificial incubation, and at 14, 28, and 39 days of incubation. Chicks and their respective eggshells were weighed on the day of hatching. Each hatch occurred in an individual compartment of the hatching machine to prevent the loss of small shell fragments.

2.3 Egg Storage

Eggs were placed on a storage chamber rack for 96 hours, with their longitudinal axes inclined at 60°, and the air cell facing upward. Turning (changing the inclination) was programmed every six hours. The temperature was maintained at 23°C, and the relative humidity at 45%.

2.4 Egg Incubation

Artificial incubation was conducted in three stages. The first stage involved a six-hour pre-incubation period, necessary for the eggs to warm from 23°C (storage temperature) to room temperature (approximately 28°C). The eggs were then placed in the egg carrier adjacent to the incubator. In the second stage, all eggs were positioned vertically in the incubator's carrier, with the air cell at the top and their longitudinal axes tilted at a 45° angle to allow left-right movement around the vertical axis. The eggs were automatically tilted every hour throughout the 39-day incubation period. Finally, the eggs were transferred to the hatching machine and monitored for up to 42 days, placed in a nesting position (horizontal longitudinal axis) in trays with individual compartments, preserving the identification code written on the shell at collection.

2.5 Egg Candling

Candling was performed individually using an opaque metal box (25 × 25 × 25 cm) containing a light bulb and a rubber lid with an elliptical opening to hold the egg. Visual inspection was carried out at the beginning of incubation and at 14, 28, and 39 days, retaining only eggs with viable embryos. Candling was used to identify infertile eggs or embryonic death, allowing non-viable eggs to be removed from the incubator. Eggs showing no signs of embryonic development were opened to determine whether they were infertile or had experienced embryonic death. Eggs that did not hatch by day 42 were opened to determine the cause.

2.6 Egg Mass Loss (EML)

The percentage of egg mass loss was calculated using the following formulas:

$$EML_{C\text{-}I}\ (\%) = \frac{\text{Mass at collection} - \text{Mass on incubation}}{\text{Mass on collection}} \times 100;$$

$$EML_{C\text{-}14}\ (\%) = \frac{\text{Mass at collection} - \text{Mass at day 14}}{\text{Mass at collection}} \times 100;$$

$$EML_{C\text{-}28}\ (\%) = \frac{\text{Mass at collection} - \text{Mass at day 28}}{\text{Mass at collection}} \times 100;$$

$$EML_{C\text{-}39}\ (\%) = \frac{\text{Mass at collection} - \text{Mass at day 39}}{\text{Mass at collection}} \times 100;$$

$$EML_{C\text{-}42}\ (\%) = \frac{\text{Mass at collection} - \text{Mass at day 42}}{\text{Mass at collection}} \times 100.$$

Where: $EML_{C\text{-}I}$: Egg mass loss from collection to incubation; $EML_{C\text{-}14}$: Egg mass loss from collection to 14 days of

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incubation; EML_{C-28}: Egg mass loss from collection to 28 days of incubation; EML_{C-39}: Egg mass loss from collection to 39 days of incubation and; EML_{C-42}: Egg mass loss from collection to 42 days of incubation.

2.7 Weighing the Chicks and Eggshells

After hatching, once the chick was fully detached from the shell and its plumage was dry, it was weighed. The shell was weighed, and both large pieces and small fragments remaining in the cage tray were collected.

2.8 Temperature, Humidity, and Air Renewal

During artificial incubation, the machine was maintained at a temperature of 36-37°C (96 to 97 °F) and a relative humidity of 25-30%. In the hatching machine, the internal temperature was maintained at 36-37°C and relative humidity at 50-60%. Automatic forced air circulation ensured uniform air renewal, avoiding significant fluctuations in temperature and humidity, and maintaining a constant oxygen supply and carbon dioxide removal.

2.9 Statistical Analysis

The results were statistically analyzed using analysis of variance (ANOVA) and descriptive statistics.

3. Results and Discussion

In this study, the results were obtained by strictly adhering to the established methodological protocol. This protocol shares similarities and differences with those employed in previous studies, as detailed below:Van Schalkwyk et al. (1999b) investigated the effect of egg exposure time in the nest prior to collection on embryonic development. Cooper (2001) and Hassan et al. (2005) recommended collecting eggs 10 to 15 minutes after laying, carefully handling them, organizing and recording the details of each egg, and labeling the shell. When necessary, eggs should be cleaned with a dry cloth, aligning with the procedures used in the present study. Rizzi et al. (2002), however, disinfected the eggs using a commercial product. Brand et al. (2012, 2020) suggested sterilization by ultraviolet light exposure for 20 minutes, followed by weighing and recording. In contrast, Mahrose et al. (2016) subjected ostrich eggs to three doses of gamma radiation under varying exposure times - unlike this study, where no disinfection was applied at any stage.

Regarding storage conditions, Deeming (1996) stored eggs at room temperature (15-24 °C) for up to 7 days, while Horbańczuk et al. (1999) used temperatures between 12-15 °C for the same duration. Gonzalez et al. (1999) stored eggs for 5 to 10 days at 18 °C with 69% relative humidity. Cooper (2001) recommended a 7-day storage period at 17-21 °C and approximately 35% relative humidity. Rizzi et al. (2002) stored eggs at 15-18 °C with and 70-75% relative humidity for up to 12 days. Ipek et al. (2003) used similar temperatures but maintained a higher relative humidity (80%) stored for 7 days. Hassan et al. (2005) varied the egg storage period before incubation from 1 to 24 days at 20 °C and 65% relative humidity. Sahan et al. (2003b), studied embryonic mortality after storing eggs for 7 days at three temperatures (16, 21 and 25 °C) with 40% relative humidity. Sahan et al. (2003a), evaluated mass loss and hatchability with storage at 16-18 °C and 35-40% relative humidity also for 7 days. Ipek & Sahan, (2006) stored eggs at 16-18 °C and 70-75% relative humidity, for 7 days. Wilson et al. (1997), used temperatures between 12-16 °C. Nahm (2001) stored eggs for 1-19 days at 15.51-15.56 °C, without humidity control and with the egg air facing downwards. Abbaspour-Fard et al. (2010) stored eggs for one week at 17-27 °C to analyze physical properties. Kontecka et al. (2011) stored eggs at 16 °C and 75% relative humidity for 7 days. Brand et al. (2007-2020) standardized storage at 17 °C and 75% relative humidity. Brassó and Komlósi (2021), in a comparative study of two Hungarian farms, adopted temperatures between 18-22 °C and 40-50% relative humidity (farm A) and 16 °C and 40% relative humidity (farm B). Moreki et al. (2023) evaluated eggs mass reduction and Haugh unit by storing at 20-25 °C for 0, 3, 6, 9, and 12 days. Brand et al. (2023) divided eggs into two groups: one stored under controlled refrigeration (17 °C and 90% relative humidity, for 1-13 days) and the other, stored for the same period, under uncontrolled room conditions to simulate typical farm storage.

In the present study, eggs were stored for four days at 23°C and 45% relative humidity. This choice was based on Fazenko (2007), who recommends temperatures between 22-25°C and high humidity (70–85%) for chicken eggs to reduce embryonic metabolism and prevent dehydration. However, in ostrich eggs, controlled dehydration is necessary to avoid oedematous chicks at hatching, justifying the lower relative humidity used in this study.

Prior artificial incubation, Horbańczuk et al. (1999) evaluated pre-warming at 25 °C for 24 hours. Deeming (1995a and 1995b) and Hassan et al. (2004) recommended pre-warming at 25 °C for 12 hours, whereas Cooper (2001) advised pre-warming at 36 °C for 4 hours. Hassan et al. (2005) simply allowed eggs to rest at room temperature for 12 hours before incubation. In this study, the adopted procedure was 6-hour pre-warming period at 28 °C.

As for incubation temperatures, Deeming (1995a and 1995b) recommended starting at 37 °C and decreasing by 0.2 °C every two days until reaching 36 °C on day 12, maintaining that until day 29, then lowering to 35.9 °C by day 39. Deeming (1996) maintained 36.5 °C, Wilson et al. (1997), used 36.4 °C, Gonzalez et al. (1999) 36.3 °C, and Cooper (2001), recommended a ranger of 36.0-36.5 °C. Relative humidity recommendations during incubation varied: 20-30% (Deeming), 15-22% (Wilson), 16-25% (Gonzalez), and; 18-22.5% (Cooper), noting that humidity may vary with altitude and could reach 60%.

Horbańczuk et al. (1999) incubated 240 ostrich eggs at 36.4 °C with four different humidity levels (50%, 40%, 30%, 25%). Gefen & Ar (2001) used 36.5 °C and 25% humidity, Nahm (2001) incubated at 36.4-37.0 °C and 18-20%humidity.

In this study, the incubation chamber was set 36-37 °C with 25-30% relative humidity. The hatching chamber maintained the same temperature range, with an increased humidity of 50-60%. By comparison, Wilson et al. (1997) and Gonzalez et al. (1999) used 36.3 °C and relative humidity of 16-25% during incubation, and 36 °C and 30% humidity during hatching. Rizzi et al. (2002) maintained 36 °C throughout but increased humidity from 25-35% during incubation to 42-45% during hatching. Sahan et al. (2003a and 2003b) used 36.5 °C and 30% humidity during incubation and 36 °C and 40% during hatching. Ipek et al. (2003) tested incubation temperatures 36.0, 36.6 and 37.2 °C for 38 days, maintained 30% humidity and increasing to 40% at hatching. Hassan et al. (2004) studied three incubation temperatures (36.5, 37.0 and 37.5 °C) with 20-30% humidity followed by 36 °C and 25-30% humidity for hatching. In another study, Hassan et al. (2005) maintained 36.5-37.0 °C and 25% humidity for 38 days, lowering the temperature to 36 °C and increasing humidity to 30% from day 39 to 44. Ipek & Sahan (2006), studying ostrich farms in Turkiye, used 36.5 °C until day 39 and 36 °C for hatching, with corresponding humidities levels of 30% and 40%. Kontecka et al. (2011) maintained 36.6 °C and 20% humidity during incubation, and 36.0 °C and 50% humidity for hatching. Brand et al. (2007-2020) consistently used 36.0 °C and 24% humidity for both incubation and hatching. Mahrose et al. (2016) after irradiating the eggs, incubated them at 36.5 °C and 25% humidity. Brassó & Komlósi (2021), reported incubation at 36.5 °C with 20-50% relative humidity.

Of the 120 eggs analyzed in this study (Table 1), 101 were fertile (84.16%), while 19 eggs were infertile (15.84%). These results surpass the fertility rates reported by several other studies. For example, Deeming (1995a) found 77.80% fertile eggs and 22.20% infertile eggs collected from nine companies in Zimbabwe. Deeming (1996) reported a mean weekly fertility rate of 74.75 ± 15.29% over a 34-week period. More (1996b) observed a median fertility of 70.00% while More (1996c) found fertility rates of 64.6% for laid eggs and 68.1% for incubated eggs. Grilli & Gallzzi (1997) recorded a fertility rate of 73.40%. In contrast, Wilson et al. (1997) reported a higher rate of 91.17% fertile eggs, exceeding the value found in this study. Van

Schalkwyk et al. (1999a) recorded a mean fertility rate of 82.30%, similar to the 80.0% reported by Nahm (2001). Rizzi et al. (2002) observed a fertility rate of 69.70%, while Ipek et al. (2003) found values of 67.4 ± 1.9%, 66.6 ± 1.3% and 67.7 ± 2.3% at incubation temperatures of 36 ºC, 36.6 ºC and 37.2 ºC respectively. Sahan et al. (2003b) reported values ranging between 70% and 73.20%, and Hassan et al. (2004) found 66.21 ± 7.6%. Ipek & Sahan, (2006) observed 69.5 ± 2.9% fertility. Dzoma & Motshegwa (2009), studying four ostrich farms, found a mean fertility rate of 76.3%, ranging from 63.5% to 89%. Lumturi & Sabah (2010), analyzing storage periods of up to 30 days, reported 57.40% fertility. Abbasfur-Fard et al. (2010) found rates of 63%, and Kontecka et al. (2011), studying females aged 7 and 5 years, found fertility 79.7 ± 2.85% and 83.5 ± 2.05%, respectively.

More (1996c) reported hatching rates of 67.00% for fertile eggs and 43.30% for all eggs analyzed. Deeming (1996) recorded a hatching rate of 31.9 ± 21.57% for fertile eggs. Wilson et al. (1997) found a 76.7% hatching rate for eggs stored for 1-4 days and 44.4% for those stored 12-14 days. Gonzalez et al. (1999), after removing contaminated and infertile eggs, achieved a hatching rate of 68.50%. Van Schalkwyk et al. (1999b) analyzing the effects of nest exposure time and pre-incubation temperature, reported hatching rates of 41.00%, 46.20%, 55.20% and 68.20%. In another study, Van Schalkwyk et al. (1999a), achieved hatching rates of 63.3%, 60.0%, and 33.8% using incubation temperatures of 36, 36.5 and 37.3 °C. Horbańczuk et al. (1999) reported 73.62% hatchability among fertile eggs and 64.9%, 72.3%, 75.0%, and 82.3% for relative humidities of 50%, 40%, 30% and 25% respectively. Nahm (2001), observed 79.20% hatchability among fertily eggs and 63.30% among total eggs, depending on storage duration (1-19 days). Rizzi et al. (2002) reported 51.50% hatchability among all eggs and 73.90% among fertile eggs. Ipek et al. (2003) found hatchability rates of 70.9 ± 1.1%, 71.6 ± 1.9% and 57.1 ± 1.7% for fertile eggs incubated at 36, 36.6 and 37.2 ºC respectively. Sahan et al. (2003a) studying eggshell thickness and pore density, reported hatchability rates 63.62%, 74.21% and 71.40% for eggs of low, medium and high shell thickness, and 40.93%, 71.80% and 80.94% for low, medium, and high pore density. Hassan et al. (2004) reported hatchability rates of 70.36 ± 6.0% at 36.5 °C, 27.31 ± 11.38% at 37.0 °C, and 23.47 ± 9.15% at 37.5 °C. Hassan et al. (2005), studying the effects of storage period and egg mass, found higher hatchability in eggs stored >5 to ≤10 days (25.90 ± 3.6%) compared to eggs stored >15 to <24 days (14.50 ± 4.7%). Additionally, higher hatchability was observed in small eggs compared to medium-mass eggs (>1,450 to ≤1,650 g), with hatchability rates of 27.3 ± 2.6%, 22.0 ± 2.0%, and 26.8 ± 3.7% in increasing mass intervals. Ipek & Sahan, (2006), reported hatchability of 68.8 ± 1.9% among fertily eggs. Dzoma & Motshegwa (2009) found overall hatchability ranging from 39.4% to 83.6% (μ = 53.8%) and mean of 65.9% and 62.9% for artificial and natural incubation, respectively, across 38,447 eggs Lumturi & Sabah (2010) achieved 52.20% hatchability among fertile eggs. Kontecka et al. (2011) reported hatchability rates of 75.6 ± 3.16% and 82.7 ± 0.35% for 7- and 5-year-old females, respectively. Among all cited studies, hatchability rates varied and were mostly lower than the rates observed in this study, which reached 83,13% for fertile eggs and 72.50% for all collected eggs.

Abbaspour-Fard et al. (2010), analyzing the physical properties of ostrich eggs, found a hatchability rate of 56%. The authors measured eggs diameters, mass, volume, density, and porosity. Although the mean pore diameter was larger in infertile eggs, the difference was not statistically significant.

Brand et al. (2011) examined the influence of water loss (ranging from <7% to >19%) on piping success and chick survival in 10,526 eggs incubated over three consecutive breeding seasons. They reported a high mean hatchability of 93.5% for eggs with embryos in the correct position-higher than the rate observed in this study. Brand et al. (2012) found mean mass loss of 12.57 ± 3.01% by day35 of incubation. Brand et al. (2020) reported a similar mean moisture loss of 12.20% over 35 days across three seasons. Mahrose et al. (2016) using gamma irradiation doses of 0.0, 0.8, 1.6, and 3.2Gy, reported respective hatching rates of 71.43%, 63.64%, 60.00% and 57.14%.

Over the 42-day incubation period in this study, embryonic mortality among fertile eggs was 13.86%. This is lower than rates reported by other researches. Deeming (1996) found 31.65 ± 22.84%, More (1996c) reported 33.00%, and Grilli & Gallazzi (1997) recorded 18.40%. Van Schalkwyk et al. (1999b) observed mortality rates between 31.8% and 59.0% under different pre-incubation temperature and nest exposure conditions. Nahm (2001) found embryonic mortality in 20.80% of fertile eggs and 16.70% of all eggs, while Rizzi et al. (2002) reported 26.10%. Ipek et al. (2003) recorded early, mid, and late embryonic mortality of 8.1 ± 1.3%, 4.8 ± 1.0%, and 16.1 ± 1.5% (at 36.0 °C); 8.3 ± 1.6%, 5.0 ± 1.2%, and 15.0 ± 1.3% (at 36.6 °C); and 15.9 ± 1.9%, 6.3 ± 1.3%, and 20.6 ± 1.7% (at 37.2 °C), respectively. Sahan et al. (2003b) reported embryonic mortality of 28.6 ± 3.2%, 32.0 ± 3.6%, and 42.9 ± 5.2%, depending on storage temperature, and 25.8 ± 2.9%, 26.7 ± 3.4%, and 24.1 ± 2.8% depending on egg positioning in the incubator. Chick mortality in this study was also lower than the values of 29.68 ± 6.22%, 58.40 ± 14.26%, and 76.53 ± 9.15% reported by Hassan et al. (2004), and the 75.7–85.5% reported by Hassan et al. (2005). Ipek & Sahan (2006) found statistically different rates for four farms: 11.7 ± 1.1%, 12.2 ± 1.5%, 8.0 ± 1.0%, and 7.7 ± 0.9% for defective position embryos; and 7.6 ± 1.4%, 8.4 ± 1.6%, 3.2 ± 0.5%, and 3.6 ± 0.4% for deformed chicks. Brand et al. (2007) reported 28.5% embryonic mortality among 37,740 eggs, while Brand et al. (2008b) recorded 29.9 ± 18.8% mortality in 969 incubated eggs. Lumturi & Sabah (2010) found 40.70% mortality among fertile eggs. Kontecka et al. (2011) reported mortality of 11.6 ± 3.00% and 4.2 ± 1.53% for 7-year-old females and 6.6 ± 0.97% and 7.9 ± 1.55% for 5-year-old. Jahantigh (2012) studied 40 inviable eggs and found that 45% contained bacterial contamination, linking fecal and soil contamination to microbial penetration and increased mortality. Brand et al. (2017) analyzed 3,472–3,484 eggs with late embryonic death (days 35–42) and found that 52.6% of embryos were in normal position, 46.5% had the head in the equatorial region, and 0.9% had inverted positioning. Moreki et al. (2023) found increased embryonic mortality with longer storage times and higher egg temperatures. Brand et al. (2023) found significantly higher mortality rates in eggs stored for only 1 day (0.38 ± 0.04%) or for 8 days or more (0.31–0.43 ± 0.04%) compared to those stored between 2 and 7 days (0.20–0.26 ± 0.04%).

In this study, all 120 eggs were placed in the incubator after 96 hours of storage, without prior candling. On day 14, the first candling was performed, revealing 19 infertile eggs (Table 1), which were removed from the process. Embryonic death was detected in two eggs at 28 days. Six more deaths occurred during days 29–39 and were detected during transfer to the hatching machine. An additional six embryos died during the hatching period, totaling 14 embryonic deaths (Table 1). This study only recorded embryonic deaths and did not analyze their specific causes.

Table 1. Number of incubated eggs, fertile and infertile eggs, hatched chicks, and embryonic deaths by female.

Parameter	Female ID														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
Incubated eggs	13	21	02	03	12	16	07	11	17	05	04	05	01	02	01	120
Fertile eggs	13	21	01	02	12	15	–	11	16	–	04	05	–	–	01	101
Infertile eggs	–	–	01	01	–	01	07	–	01	05	–	–	01	02	–	19
Hatched chicks	10	18	01	02	12	11	–	10	15	–	04	03	–	–	01	87
Embryonic deaths	03	03	–	–	–	04	–	01	01	–	–	02	–	–	–	14

Source: Research data (2025).

Analysis of the data in Table 2, based on the 87 eggs that hatched, revealed a mean egg mass from the beginning (collection at the nest) to hatching (42 days) of 1,333.49 ± 171.04 g and 1,068.80 ± 154.74 g, respectively, with a mean mass loss of 264.69 g over the course of the process. The mean chick mass at hatching was 815.56 g, representing 61.15% of the initial egg mass.

More (1996c) reported working with eggs whit a mean mass of 1,301.9 ± 113.2 g, with a mean mass loss of 15.5% from collection to day 39 of incubation. However, the study did not specify the temperature or relative humidity used in the incubator. Wilson et al. (1997) used eggs with a mean mass of 1,473 g and observed a mass loss of 17.4% up to day 38 of incubation, under controlled temperatures for storage (12–16 °C), incubation (36.4 °C), and hatching (36.0 °C), with relative humidity maintained at 15–22% during incubation and 20–40% during hatching.

Horbańczuk et al. (1999), studying different relative humidity conditions, used eggs with a mean mass of 1,621.0 ± 104.5 g. Gonzalez et al. (1999) examined factors affecting hatchability using eggs ranging from 1,450 to 1,650 g, reporting mass losses between 11.4% and 14.7% during incubation. Nahn (2001), without specifying the initial egg mass, reported mass losses during incubation ranging from 12.3 ± 1.01% to 16.2 ± 0.77%. Gefen & Ar (2001) found a mean initial egg mass of 1,440.60 ± 157.50 g (n = 34) in a study on gas exchange through shell pores, with daily water losses ranging from 0.27% to 0.36% of the initial mass.

Superchi et al. (2002) reported a mean egg mass of 1,444 g, which varied during the laying period. Rizzi et al. (2002) found a mean initial egg mass of 1,558.00 ± 167.00 g (n = 89). Ipek et al. (2003), studying the effects of increasing incubation temperatures (36.0, 36.6, and 37.2 °C), used eggs with mean masses of 1,420.2 ± 42.7 g, 1,409.2 ± 38.8 g, and 1,427.5 ± 53.9 g, respectively. Sahan et al. (2003a) worked with eggs a mean mass of 1,618 ± 14.05 g. Hassan et al. (2004) reported an overall mean mass of 1,520.04 ± 11.47 g. Bunter & Cloete (2004), in a study on genetic parameters for egg mass, observed a mean of 1,429.60 ± 141.00 g. Hassan et al. (2005) reported egg masses ranging from 1,501.70 ± 15.20 g to 1,588.30 ± 30.90 g. Ipek & Sahan (2006) found eggs with a mean mass of 1,439 ± 36.6 g.

In the study by Lumturi & Sabah (2010), the mean initial egg mass was 1,572.55 ± 140.06 g. Brand et al. (2008a), analyzing factors affecting incubation characteristics, worked with eggs averaging 1,424 ± 0.134 g. Brand et al. (2008b), investigating the genetic relationship between mass loss and embryonic death, used eggs with a mean mass of 1,425 ± 107 g. Abbaspour-Fard et al. (2010) used eggs weighing between 1,300 g and 2,100 g. Kontecka et al. (2011) examined laying and hatching characteristics in females of different ages, reporting mean egg masses of 1,456.7 ± 39.06 g for 7-year-old females and 1,405.9 ± 55.80 g for 5-year-old. Brand et al. (2013), in a study using candling images, presented a mean mass of 1,389 ± 112 g for the 120 eggs analyzed. Brassó & Komlósi (2021), evaluating quality parameters of Hungarian ostrich eggs, found a mean mass of 1,432.64 ± 9.44 g. Moreki et al. (2023) reported a mean egg mass of 1,223.13 ± 51.73 g.

In this study, egg mass ranged from 1,108 g to 1,772 g, demonstrating values similar to those reported in other regions of the world, as discussed above.

Horbańczuk et al. (1999), studying different relative humidity levels, found mass losses during incubation ranging from 10.18% to 13.51%. Gefen & Ar (2001) reported mass losses over the full 42-day incubation period ranging from 11.30% to 15.10%. Rizzi et al. (2002) reported a mean mass loss of 14.01 ± 3.6% at 38 days of incubation. Ipek et al. (2003), in their study on incubation temperatures, observed mass losses of 12.4 ± 0.2%, 13.7 ± 0.3%, and 15.5 ± 0.5% for incubation at 36.0, 36.6, and 37.2 °C, respectively.

Sahan et al. (2003a), analyzing eggshell thickness and porosity, found greater mass loss (13.03%) in eggs with low shell thickness compared to those with intermediate (11.22%) and high shell thickness (10.36%). Hassan et al. (2004) observed mass losses at 38 days of incubation of 12.14 ± 0.25%, 12.52 ± 0.33%, and 12.66 ± 0.36% at temperatures of 36.5, 37.0, and 37.5 °C, respectively. Ipek & Sahan (2006) reported mass losses of 12.3 ± 0.4% at day 39. Brand et al. (2008a) observed mass losses of 12.8 ± 3.10%, while Brand et al. (2008b) reported mean losses of 13.1 ± 2.2% by day 35.

Lumturi & Sabah (2010) recorded a two-week mass loss of 5.26%. Brand et al. (2011) found mass loss ranging from <7% to >19% by day 35. Kontecka et al. (2011) reported mean mass losses of 15.9 ± 0.65% in eggs from 7-year-old females and 16.1 ± 0.75% in 5-year-old. Brand et al. (2013) reported water losses ranging from 4.6% to 13.0% on day 21 and from 7.4% to 20.0% on day 35. Brassó & Komlósi (2021) reported a mean mass loss of 14.72% by day 38.

In this study, the mean mass loss for the 87 eggs analyzed up to day 39 was 15.18 ± 3.44%, aligning with the findings of the aforementioned authors. The mean total mass loss over the 42-day incubation period was 19.88 ± 5.21%.

Table 2. Mean egg and chick masses from collection to hatching, percentage of chick mass relative to initial egg mass, total mass loss during incubation, and range of values for each variable.

Stage	n	Mean ± SD (g)	Mass (%)	Mass loss (%)	Range (g)
Egg at collection	87*	1,333.49 ± 171.04	100	-	1,108 – 1,772
Start of incubation	87	1,322.97 ± 170.57	99.21	0.79 ± 0.39	1,098 – 1,760
14 days of incubation	87	1,256.37 ± 163.46	94.21	5.79 ± 1.67	1,012 – 1,682
28 days of incubation	87	1,191.01 ± 158.66	89.27	10.69 ± 2.67	936 – 1,620
39 days of incubation	87	1,130.99 ± 152.55	84.35	15.18 ± 3.44	868 – 1,566
At hatch (42 days)	87	1.068.80 ± 154.74	80.12	19.88 ± 5.21	797 – 1,536
Chick at hatch	87	815.56 ± 129.23	61.15	-	595 – 1,206
Eggshell	87	253.24 ± 32.61	18.98	-	202 – 338
Chick/egg at incubation (%)	87	61.15 ± 4.82	-	-	49.30 – 68.78

* Note: Data refer exclusively to the 87 eggs that completed the full incubation process.
Source: Research data (2025).

When analyzing the mean egg mass per female individually, it was observed that the percentage of mass loss was not directly proportional to the initial egg mass. As shown in Table 3, female. 5 had the highest mean egg mass at collection (1,674.83 ± 60.16 g) and a loss of 17.39 ± 2.97% by the 39th day of incubation. In contrast, female 2, which had the lowest mean egg mass (1,206.47 ± 40.07 g), exhibited a slightly higher loss (17.44 ± 5.52%) over the same period.

The greatest percentage of mass loss (26.08 ± 3.53%) was observed in eggs from female 11, whose mean initial mass was 1,411.00 ± 28.02 g. Conversely, the lowest mass loss (15.85 ± 3.59%) occurred in eggs from female No. 9, which had a mean initial mass of 1,235.60 ± 54.10 g. These results demonstrate that higher initial egg mass does not necessarily result in greater percentage mass loss, even when all eggs are incubated under the same temperature and relative humidity conditions.

Deeming (1995a) and Blood et al. (1998) emphasized the importance of water loss during incubation, reporting that hatchability was higher when mass loss ranged between 8–18% or 10–20%, and lower when eggs experienced either insufficient or excessive water loss. Nahm (2001) also stated that hatchability was negatively affected when mass loss exceeded 20% during incubation.

However, the data obtained in the present study contrast with the findings of Deeming (1995a), Blood et al. (1998), and Nahm (2001). Here, 21 fertile eggs lost more than 18% of their initial mass by day 39 of incubation, and by day 42, 42 eggs (48.27% of the total) had lost over 20% of their initial mass and still hatched successfully.

Table 3. Mean egg mass per female during incubation, chick mass at hatch, total mass loss, and respective ranges and amplitudes for egg mass and loss.

Parameter	Female 1 (n = 10)	Female 2 (n = 18)	Female 5 (n = 12)	Female 6 (n = 11)	Female 8 (n = 10)	Female 9 (n = 15)	Female 11 (n = 4)
Initial egg mass (g)	1,310.60± 60.74	1,206.89± 43.37	1,674.83± 60.17	1,213.45± 44.16	1,484.30± 68.18	1,235.60± 54.11	1,411.00± 28.02
Start of incubation (g)	1,297.60± 59.85	1,195.67± 39.28	1,663.92± 59.05	1,203.45± 43.61	1,474.40± 67.26	1,225.87± 53.39	1,402.00± 27.28
14 days of incubation (g)	1,229.80± 66.29	1,145.33± 34.16	1,586.67± 59.41	1,121.27± 54.64	1,379.40± 78.55	1,175.60± 55.05	1,334.50± 33.04
28 days of incubation (g)	1,154.00± 6.88	1,092.78± 35.11	1,511.17± 62.66	1,046.45± 58.55	1,297.60± 95.56	1,120.13± 55.04	1,270.50± 53.58
39 days of incubation (g)	1,100.00± 79.72	1,044.56± 40.29	1,442.08± 62.35	984.18± 59.36	1,236.10± 89.90	1,065.80± 52.72	1,186.50± 45.03
At hatch (42 days) (g)	1,011.60± 54.01	996.83± 80.29	1,384.00± 82.17	932.36± 78.92	1,145.00± 99.28	1,025.73± 67.57	1,042.75± 48.30
Chick mass at hatch (g)	784.60± 49.35	764.11± 71.13	1,073.17± 74.83	700.73± 67.74	854.90± 90.08	784.73± 60.01	773.50± 61.46
Eggshell mass (g)	227.00± 12.87	232.42± 13.53	310.83± 12.25	231.64± 14.44	291.00± 13.97	241.00± 12.54	269.25± 20.90
*Mass loss (%)	22.80± 2.57	17.44± 5.53	17.40± 2.98	23.27± 4.26	22.86± 4.41	16.96± 4.60	26.08± 3.54
Egg mass range (g)	1,182–1,406 (224g)	1,136–1,302 (166g)	1,566–1,772 (206g)	1,108–1,256 (148g)	1,342–1,614 (272g)	1,128–1,332 (204g)	1,376–1,442 (66g)
Mass Loss range (%)	20.52–26.05 (5.53%)	12.56-29.49 (16.93%)	13.32-28.85 (9.53%)	17.83-29.76 (11.93%)	16.60-30.67 (14.07%)	11.52-28.19 (17.67%)	21.87-30.52 (8.65%)
Hatching rate per Female (%)	76.92	85.71	100.00	73.33	90.90	93.75	100.00

Note: Females with fewer than four incubated eggs were excluded from this summary.
* *Mass loss calculated as: (initial egg mass – [chick mass + shell mass]) / initial egg mass × 100.*
Source: Research data (2025).

The increase in age of galliforms birds leads to enlargement of ovarian follicles and greater yolk deposition, thereby increasing egg size and mass (Almeida et al., 2006). According to Rocha et al. (2008), the total amount of yolk produced by hepatic synthesis remains consistent regardless of the bird's age, but deposition occurs in a smaller number of follicles, explaining the tendency toward increased yolk volume. Vieira & Moran (1998) also noted that the increase in egg size and mass in chickens follows a gradual upward trend of approximately 0.47–0.54 g per week.

In the laying sequence of females in this experiment (Figure 1, panels A to G), a pattern of fluctuations in egg mass was observed within the same laying cycle, resulting in a wide amplitude of egg masses (Table 3). This differs from the trend typically seen in galliforms birds, suggesting that the glandular secretion of internal and external membranes, albumen, and shell components along the oviduct during ostrich egg formation occurs in non-uniform quantities, even within eggs from the same female.

The most frequent mass loss range was 20–22% (16 eggs), followed by 14–16% (14 eggs). According to Christensen et al. (1996), to achieve 15% water loss during incubation, the ideal relative humidity should be below 25%. Most cited studies report egg mass loss up to 39 days of incubation. In the present study, mass loss up to day 39 ranged from 10.14% (egg 06 from female 6) to 25.17% (egg 02 from female 5). The incubator's relative humidity was maintained between 25% and 35%, indicating that relative humidity levels above 25% were still capable of inducing mass losses greater than those reported by Christensen et al. (1996).

Natural incubation of ostrich eggs ranges from 39 to 42 days (Swart et al., 1987; Bertram, 1992). However, based on allometric predictions by Rahm & Ar (1974), the expected incubation period for a 1,500 g ostrich egg would be 59 days (ranging from 48 to 73 days). Under conditions described by Swart et al. (1987), a 59-day incubation could result in approximately 24% water loss - considerably higher than the ~15% typically observed in other avian species. Such water loss would significantly reduce hatchability in ostrich eggs (Ar et al., 1996).

The hatch rate in this study was 86.13%, which is high and exceeds values frequently reported in the literature. It was observed that 22.77% of hatched eggs had mass losses exceeding 24%, and 28.71% of the eggs hatched with losses between 18% and 24%. Altogether, 51.48% of the hatched eggs had mass losses between 18% and 32%, considered high by literature standards. Despite this, hatchability was not compromised, suggesting that the relative humidity used did not adversely affect embryonic development.

The relatively short incubation duration of ostrich eggs (42 days), compared to other bird species, is likely an adaptation to arid environmental conditions in their natural habitat. The embryo may avoid dehydration by hatching earlier, and because ostriches are precocial, they use relatively little yolk during incubation (Gefen & Ar, 2001). Stewart (1996) emphasized that air circulation in the incubator is crucial to ensuring uniform temperature, humidity, and gas exchange (O_2 and CO_2). Gonzalez et al. (1999) concluded that shell thickness and pore characteristics influence hatchability, with excessive or insufficient porosity impairing gas exchange and reducing hatch success.

The range of egg mass loss observed in this study (19.15%) was greater than in most previous publications. This suggests that the variability in mass loss was likely determined by physical shell characteristics—such as thickness, diameter, and number of pores—which differed not only among females but also among eggs from the same female. Additionally, continuous airflow around incubated eggs, as noted by Stewart (1996), likely contributed to maintaining proper oxygen and carbon dioxide levels. Despite the broad variation in mass loss, good hatchability was achieved, likely due to efficient gas exchange, supporting the findings of Gonzalez et al. (1999), who demonstrated that medium-sized eggs had the highest hatchability (77.10%) compared to small (50.00%) and large eggs (68.20%).

Using the classification by Gonzalez et al. (1999) - large (>1,650 g), medium (>1,450 g and ≤1,650 g), and small (≤1,450 g) - this study found a 100% hatch rate for both large and medium eggs, while small eggs had a hatch rate of 67.00%. When considering only fertile eggs, the overall hatch rate was 82.71%, differing from the findings of the cited authors.

Analyzing each egg individually (Table 04), it was noted that the eggs with the lowest and highest masses from each female showed similar percentages of mass loss. For example, female 1 had eggs weighing 1,182 g and 1,406 g, with respective losses of 25.55% and 25.75%. Females 2, 5, 9, and 11 exhibited the same pattern. However, females 6 and 8 deviated: female 6 showed higher mass loss in her lowest-mass egg, while female 8 had greater loss in her highest-mass egg.

Table 4. Percentage of mass loss during incubation for the smallest and largest eggs laid by each ostrich female.

Ostrich female	Lowest egg mass (g)	Mass loss (%)	Highest egg mass (g)	Mass loss (%)
1	1,182.00	25.55	1,406.00	25.75
2	1,136.00	28.35	1,302.00	29.49
5	1,566.00	14.30	1,772.00	13.32
6	1,108.00	28.07	1,256.00	17.83
8	1,342.00	19.37	1,614.00	25.03
9	1,128.00	14.01	1,332.00	14.56
11	1,376.00	25.87	1,442.00	26.07

Source: Research data (2025).

Figure 1 presents the individual egg masses at the time of nest collection and their corresponding mass losses from incubation to hatching, illustrating that mass loss is not directly proportional to initial egg mass. In Figure 1A, for instance, egg 07 had the lowest initial mass and one of the highest mass loss percentages. Similarly, eggs 01 and 03 in Figure 1B, which also had lower initial masses, experienced proportionally greater mass loss. The same trend was observed for eggs 08 and 09 in Figure 1D and egg 07 in Figure 1F.

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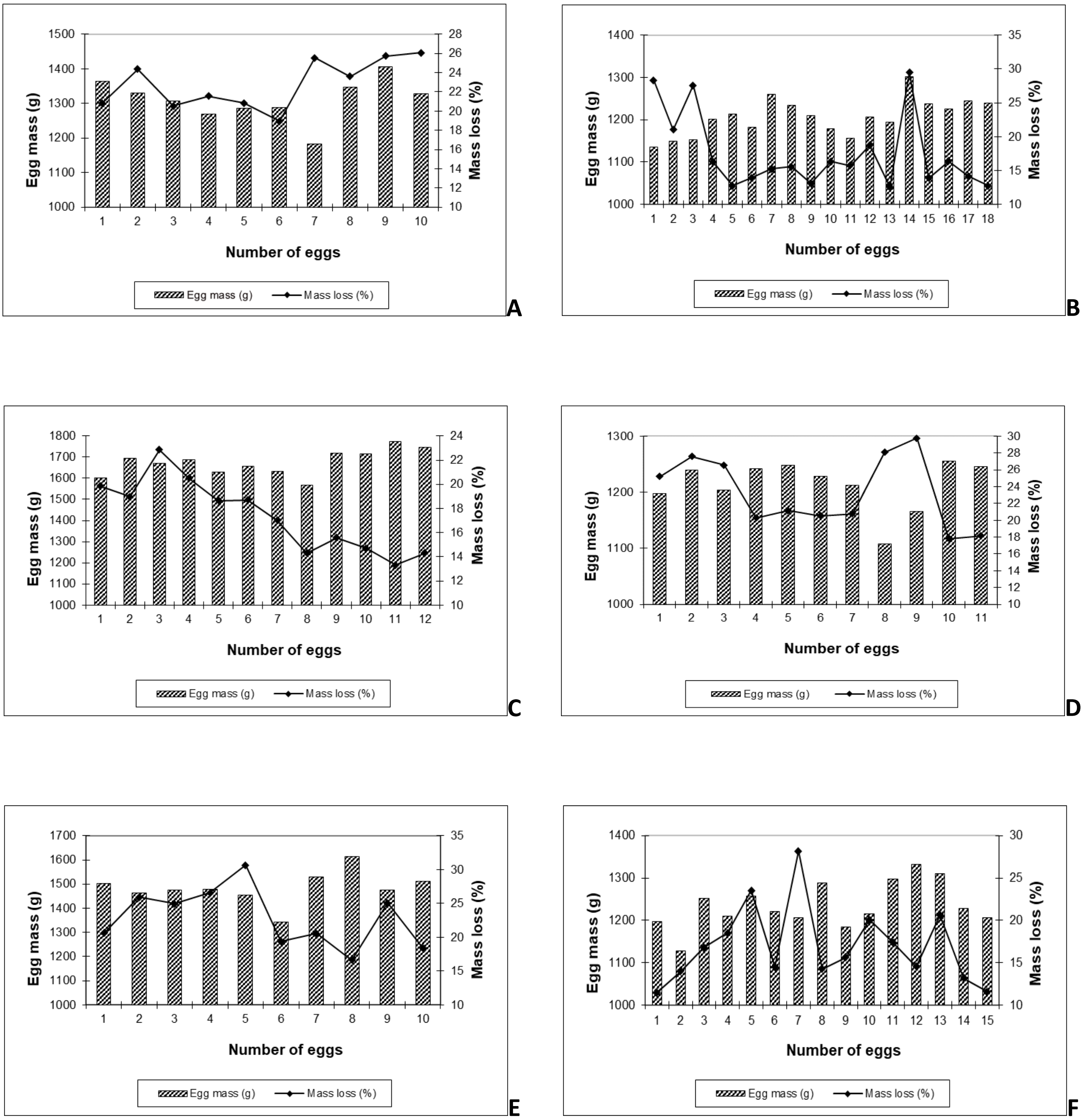
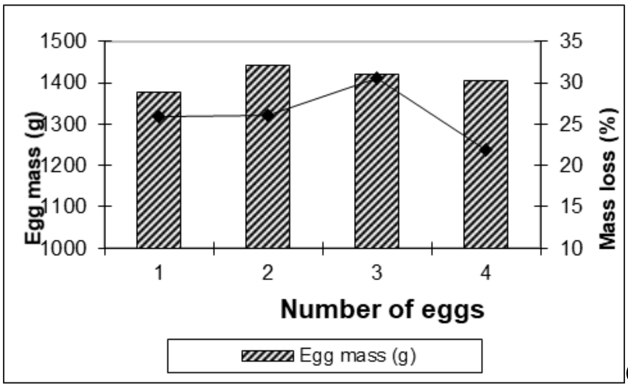
Figure 1. Individual egg masses at collection (g) and corresponding percentage mass loss at chick hatching for females 1, 2, 5, 6, 8, 9, and 11.





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The percentage of egg mass loss in this study varied widely, from 11.52% (egg 01 from female 6) to 30.67% (egg 05 from female 5), as shown in figure 1C and 1D. There was also noticeable variation in mass loss between eggs from the same female, indicating a lack of uniformity in mass loss despite standardized bird management, paddock environment, diet, and incubation protocol. Female 1 (Figure 1A) had the highest frequency (4 eggs) of mass loss between 20% and 22%, with a hatch rate of 76.92%. Female 2 (Figure 1B) most frequently (6 eggs) showed mass losses between 12% and 14%, with a hatch rate of 85.71%. Eggs from female 5 (Figure 1C) exhibited two peak intervals of mass loss (4 eggs each): 14–16% and 18–20%, and achieved 100% hatchability. Female 6 (Figure 1D) had four eggs with the highest mass losses (20–22%) and experienced losses as high as 29.76%, with a hatch rate of 73.33%. Female 8 (Figure 1E) had three eggs with losses between 24% and 26%, and a hatch rate of 90.90%. Female 9 (Figure 1F) showed the widest range of mass loss, from 11.52% to 28.19%, and had a hatch rate of 93.75%. Female 11 achieved a 100% hatch rate. Notably, egg 03 from female 11 (Figure 1G) had the second-highest mass loss (30.52%) among all eggs in this study.
Source: Research data (2025).

4. Conclusion

Several experimental protocols for ostrich egg incubation have been described in the scientific literature, addressing multiple factors that influence hatching success. These studies include procedures ranging from egg collection at the nest and shell surface cleaning to storage in chambers with controlled temperature and relative humidity for varying duration.

Previous studies have correlated physical parameters—such as egg volume, mass, chick mass at hatch, shell thickness and weight, and the number and size of shell pores—with hatching rates. Other factors examined include embryo positioning within the egg, the genetic background of the birds, breeder diet, and environmental conditions such as latitude, longitude, climate, and seasonality. Proper management of incubation and hatching equipment is also critical, considering variables such as egg orientation (vertical, horizontal, or inclined), automatic turning mechanisms, and the rate of mass loss during incubation, which may be either insufficient or excessive. Additionally, the occurrence of microbial contamination and the hatching of dehydrated or oedematous chicks are important considerations.

In the incubation protocol tested in this study, egg mass loss was observed to be higher and different from that reported in other studies; however, it resulted in a higher hatching rate. Fertility, infertility, and embryonic mortality rates also showed more favorable outcomes than those reported in previously cited studies.

Ostrich farming in captive systems requires further research to establish appropriate feeding and nutritional strategies that optimize reproductive performance. The intensification of breeding also necessitates monitoring additional variables, such as sperm quality assessments in males, mating frequency, photo-period regimes (lighting programs adjusted to the daily light duration in each region), and the evaluation of serum hormone levels in both males and females. These measures aim to maximize annual egg production per female and, consequently, increase the output of day-old chicks.

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