SCIENTIFIC ARTICLE

Embryonic development of quail eggs (*Coturnix coturnix japonica*) in a homemade incubator

Desenvolvimento embrionário de codornas (*Coturnix coturnix japonica*) em incubadora artesanal

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Abstract

Knowledge of the embryonic development of species such as quail is important for our understanding of its production and reproduction. Quail provides a practical solution to the animal protein shortage problem in developing countries and is an excellent alternative to chicken (Shanaway, 1994). This study evaluated quail embryonic viability by comparing the main morphological changes that occur over the development in a homemade incubator with previous data; the incubator was built using easily accessible and low-cost materials, which allows small producers and communities to develop quail farms as an activity to increase income or even produce a source of animal protein for the community in the Brazilian Amazon region. The homemade incubator measured 40 cm × 42 cm × 32 cm and was constructed with wooden boards, a digital thermostat, and an incandescent lamp. A total of 24 fertile eggs were incubated at a temperature of 37.5 °C and 60% relative humidity; age "0" was set at the start of incubation. Two eggs were opened on each day of development, starting from day 5 to day 16 of incubation. After gently opening the eggs, the embryos were removed, separated from the placenta and amniotic fluid, washed with running water, and weighed on a digital scale (SHIMADZU AUY 220 brand and UNI BLOC model). Crown-rump length and other measurements were performed with a digital caliper and the morphological structures were observed using a stereomicroscope (NOVA brand and NOVA ZTX-E model). All eggs opened contained live embryos, indicating that the homemade incubator temperature, relative humidity, and manual turning were adequate to maintain the quail's viability. The morphometric data of the embryos at different developmental time points were similar to those described in the literature. Embryos weighed 0.0069 g at day 5 and 4.7863 g at day 16, and the crown-rump length (CRL) means were 0.368 cm and 3.657 cm, respectively. Weight and CRL increased by 0.434 g and 2.593 cm per day of incubation, respectively. In conclusion, after 16 days of incubation in a homemade incubator, the embryos presented all phases of the development cycle, with appropriate development of weight and height based on the days of incubation without anomaly or external interference. These findings demonstrate that the homemade incubator does not alter the embryonic development of quail embryos in the Brazilian Amazon region.

Keywords: morphology, embryo, incubation, Brazilian Amazon region.

Resumo

O conhecimento do desenvolvimento embrionário de espécies como a codorna é importante para nosso entendimento de sua produção e reprodução. A codorna fornece uma solução prática para o problema de escassez de proteína animal nos países em desenvolvimento e é uma excelente alternativa ao frango (Shanaway, 1994). Este estudo avaliou a viabilidade embrionária de codornas comparando as principais alterações morfológicas que ocorrem ao longo do desenvolvimento em uma incubadora caseira com dados anteriores; a incubadora foi construída com materiais de fácil acesso e baixo custo, o que permite que pequenos produtores e comunidades desenvolvam criações de codornas como atividade para aumentar a renda ou mesmo produzir uma fonte de proteína animal para comunidades da Amazônia brasileira. A incubadora caseira media 40 cm × 42 cm × 32 cm e foi construída com tábuas de madeira, termostato digital e lâmpada incandescente. Um total de 24 ovos férteis foram incubados a uma temperatura de 37,5 °C e 60% de umidade relativa; idade "0" foi definida no início da incubação. Dois ovos foram abertos

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a cada dia de desenvolvimento, começando do dia 5 ao dia 16 de incubação. Após a abertura cuidadosa dos ovos, os embriões foram retirados, separados da placenta e do líquido amniótico, lavados em água corrente e pesados em balança digital (marca SHIMADZU AUY 220 e modelo UNI BLOC). O comprimento cabeça-cauda e demais medidas foram realizadas com paquímetro digital e as estruturas morfológicas foram observadas com auxílio de estereomicroscópio (marca NOVA e modelo NOVA ZTX-E). Todos os ovos abertos continham embriões vivos, indicando que a temperatura da incubadora caseira, a umidade relativa e a viragem manual foram adequadas para manter a viabilidade das codornas. Os dados morfométricos dos embriões em diferentes momentos do desenvolvimento foram semelhantes aos descritos na literatura. Os embriões pesaram 0,0069 g no dia 5 e 4,7863 g no dia 16, e as médias do comprimento cabeça-cauda (CRL) foram de 0,368 cm e 3,657 cm, respectivamente. Peso e CRL aumentaram 0,434 g e 2,593 cm por dia de incubação, respectivamente. Em conclusão, após 16 dias de incubação em incubadora caseira, os embriões apresentaram todas as fases do ciclo de desenvolvimento, com desenvolvimento adequado de peso e altura com base nos dias de incubação sem anomalia ou interferência externa. Esses achados demonstram que a incubadora caseira não altera o desenvolvimento embrionário de embriões de codorna na Amazônia brasileira.

Palavras-chave: morfologia, embrião, incubação, Amazônia brasileira.

Introduction

Quail production requires a holistic evidence-based approach, which will provide answers to each stage of embryonic development. Understanding these stages makes it possible to obtain viable and quality quail eggs and meat, an excellent alternative to other animal protein consumption (Araújo et al., 2015)

Currently, artificial incubation is an important factor in the development of aviculture and represents the interaction between science and food production (Menezes et al., 2020).

Additionally, quail species are used as a suitable intermediate host for biological experimental infections (Rodrigues et al., 2012) and toxicological studies (Magnoli et al., 2013).

In 2020, the Brazilian quail population was 16,512,169 birds. The production of eggs in the same year amounted to 295,904 (x1000) dozen eggs, generating a value of R$ 374,567,000.00 (Instituto Brasileiro de Geografia e Estatística, 2020).

In a large-scale poultry production, artificial incubation is advantageous. Storage of fertile eggs before hatching is a critical factor in production (Araújo et al., 2015). Storage is one of the common procedures on farms to reduce transport costs; however, sufficient eggs must be present to fill machines and synchronize hatching (Fasenko, 2007).

Egg incubation may be associated with environmental conditions and hormonal changes (Thierry et al., 2013). Conditioned rooms and adequate storage directly influence the success of incubation (Menezes et al., 2020). Extended storage increases mortality and reduces hatching rates (Elibol et al., 2002). Moreover, the storage temperature of fertile eggs influences embryonic mortality (Mahmud et al., 2011).

Japanese fertile quail eggs stored for longer periods should be at 14ºC to guarantee a better physical quality of the progeny (Araújo et al., 2015). Room temperature (26.5 °C) is not suitable for storage exceeding 72 hours. Fertile eggs are incubated for 16 days, the ideal temperature is 37.5 °C, and the air relative humidity should remain between 55 and 65% (Pedroso et al., 2006).

Quail embryology has been described in 46 stages. The appearance of several structures indicate the stage of normal development in hours/days of incubation, such as the elongated primitive streak - 4th stage (18-19 h.), notochord - 5th stage (19-22 h) head fold apparent - 6th stage (23–25 h), somite number evident - 1 (7th stage, 23-26 h), 4 (8th stage, 29 h), 7 (9th stage, 33 h), 10 (10th stage, 38 h), 13 (11th stage, 45 h), 16 (12th stage, 49 h), 19 (13th stage, 52 h), 22 (14th stage, 53 h), 24-27 (15th stage, 55 h), 26-28 (16th stage, 56 h) and 29-32 (17th stage, 64 h); other structures such first and second branchial arches visible (50 to 53 h) third branchial arch defined (50–55 h), the first appearance of the wing bud (51–56 h), leg bud visible and the wing buds expanded slightly (52 and 64 h, respectively), presence of the allantois and closed amnion (72 h) (Ainsworth et al., 2010)

The count in days starts from 72 h and describes the maxillary process and unpigmented eyes (3 d), pigmented eyes, limb buds equal in width and length and limb buds greater in length than width (4 d), elbow and knee joints distinct (4.5 d), demarcation of toes (4.5–5 d), presumptive region

of beak identifiable (5 d), beak outgrowth distinct (5.5 d), bend at the wing elbow visible. No egg tooth apparent (5.5 - 6 d), wing curl (6 d), one to two scleral papillae and egg tooth (6–6.5 days), 1–2, 6, 6-8, and 13 scleral papillae (6.5, 6.5, 7, 7 d, respectively), eyelids (8 d), beak with 1.2, 1.5, 2, 2.3, 2.6, 3, and 3.5 mm and third finger 3, 2, 4.7, 6.1, 8.6, 9.4, 10.8, and 11.9 mm (10, 12, 14, 16, 17, 18, and 19.5 d, respectively), black pigmentation on the sides of the skull and brown in the lumbosacral region (12 d), hair follicles (10.5–11 d), hair follicles throughout the eye region (14 d), white feather follicles along the entire length of the embryo and around the eyes (15 d), pigmentation on the toes at 16 days, when hatching occurs (Ainsworth et al., 2010). Readers are referred to the original report for an accurate description of the stages.

Brazilian quail production is justified by the small size of the birds, low cost, and reduced time to reach sexual maturity. Quail is a practical solution to the animal protein shortage in developing countries and is an excellent alternative to chicken (Shanaway, 1994).

This study evaluated quail embryonic viability by comparing the main morphological changes that occur over the development in a homemade incubator with previous data; the incubator was built using easily accessible and low-cost materials, which will allow small producers and communities to develop quail farms as an activity to increase income or produce a source of animal protein for the community in the Brazilian Amazon region.

Material and methods

The homemade incubator measuring 40 cm × 42 cm × 32 cm was built using six wooden boards (12 mm each), a digital thermostat w1209, an incandescent lamp of 100 W, hex screen m1/2xf24×1.5-m, and acrylic window 15 cm x 10 cm (Figure 1).

Figure 1. Homemade incubator.

The experiments were conducted at the Laboratório de Morfofisiologia Animal of the Universidade Federal do Oeste do Pará. The homemade incubator contained 80 eggs (Figure 1). The thermostat was regulated to maintain the temperature between 37.5–38.0ºC; a water contained placed in the incubator to provide humidity was sanitized and refilled every 3 days or when low levels of water were observed. To provide gas exchange, a small hole measuring 8 × 8 cm was created in the upper part of the incubator. The eggs, which were placed under a screen, were observed through an acrylic window and turned manually three times a day until day 16.

Fertile eggs from the Poultry II module of the Animal Science course were collected, transported, and disinfected with a towel soaked in running water and identified with an X. The initial conditions of the experiment were 35.2°C with 80% relative humidity, typical of the Brazilian Amazon region.

Twenty-four hours prior to the experiment, the incubator was switched on. The following day, a total of 24 fertile eggs were incubated at 37.5°C with 60% relative humidity; age "0" was set at the start of incubation.

During the experiment, two eggs were opened each day from day 5 to day 16 of incubation. After gently opening the eggs, the embryos were removed, separated from the placenta and amniotic fluid, washed with running water, and weighed on a digital scale (SHIMADZU AUY

220 brand and UNI BLOC model). Crown-rump length and other measurements were performed with a digital caliper and the morphological structures were observed using a stereomicroscope (NOVA brand and NOVA ZTX-E model).

The main external structures at each stage of embryonic development were observed, and the specimens were then fixed in a flask containing 10% formaldehyde and stored in the Laboratório de Morfofisiologia at Universidade Federal do Oeste do Pará. The external parts including morphological characteristics of the eye, beak, limbs (position, presence of number of fingers), feather follicles, and external genitalia were observed and photodocumented for comparison with other studies. The criteria used to describe the external morphology were the same as those used for Japanese quail embryos previously (Ainsworth et al., 2010; Ramteke et al., 2013). Data were analyzed using Microsoft Office 2013 Excel software to obtain the mean, standard deviation, and regression graphs. Regression analysis of weight and height in relation to the period of development were performed using the Sisvar 5.6 Software. The use of animals in this study was approved by the Institutional Commission for Ethics for the Use of Animals of the Universidade Federal do Oeste do Pará (Protocol number 10012-2017).

Results

All eggs contained live embryos, which indicates that temperature and relative humidity of the homemade incubator and manual turning were adequate to maintain the viability of the quail; morphometric data of the embryos at different developmental stages are shown in Figure 2 and described in Table 1. Some of the highlighted external morphological areas are shown in Figure 3. Embryos with 5–6 days of incubation (Figure 2A-2B) have a "C" curve shape, with the maximum curvature occurring at 6 days. The cephalic region appeared larger than the caudal region, and the eyelids and eyes were not yet homogeneously pigmented (Figure 3A). Heartbeat, beak in formation, and presence of somites were observed (Figure 3B); after seven days of incubation, the eyes became observably more homogeneously pigmented (Figure 3C). At this stage, the skin was thin and transparent, allowing the observation of a dark line along the length of the dorsal region. The heart was located in the cervical region, and the thoracic limb buttons and pelvis were clearly visible, with rounded ends (Figure 3C). Embryos on day 8 of incubation presented eyelids in the early stages of growth on the surface of the eyeball (Figure 2D). On day 9 of incubation, the heart (Figure 3D) showed black and brown pigmentation

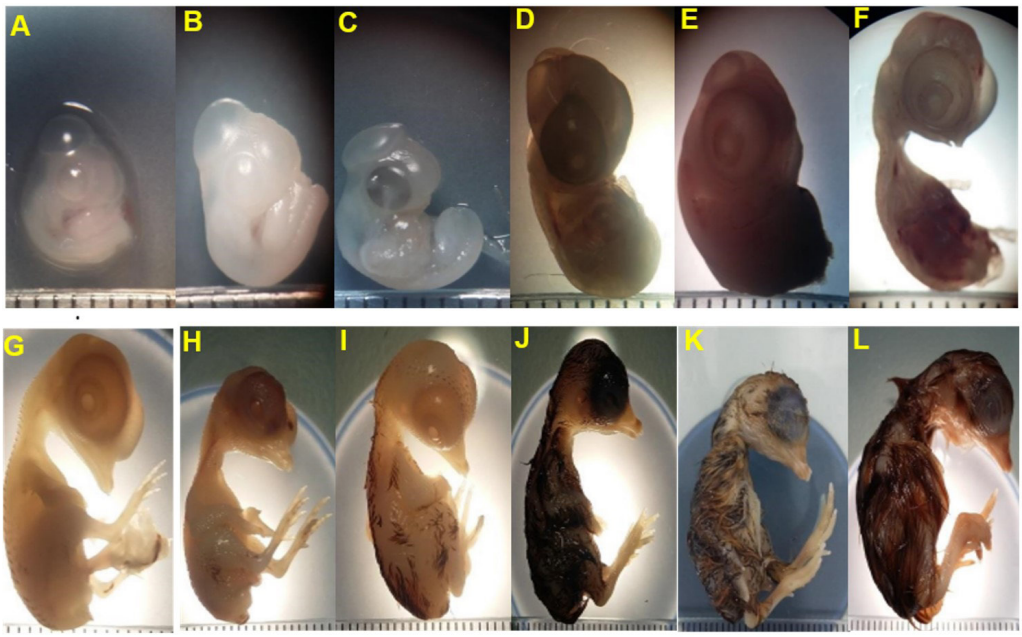

Figure 2. Quail embryos external morphology development.
Note: Quail embryos with 5(A); 6(B); 7(C); 8(D); 9(E); 10(F); 11(G); 12(H); 13(I); 14(J); 15(K) and 16(L) days of incubation.

Table 1. Description of key features of quais embryonic development.

Days of incubation	Highlighted external morphological descriptions
5º	The cephalic region is larger than the caudal region, the eyes are not totally pigmented and somites are visualized.
6º	Maximum "C" flex and the yolk sac are clearly visible, and the heartbeat is noted, just like the formation of the fore and hind limbs.
7º	Totally pigmented eyes and beak formation. Very transparent skin allows visualization of rounded thoracic and pelvic limbs.
8º	Quite larger eyes, strongly pigmented. Pelvic limbs and developing digits. The heart in the dorsal region is well visualized.
9º	Beginning of beak formation, well-developed limb buds and presence of eyelids.
10º	Presence of the beak and mandible. "Egg Tooth" appears.
11º	First feathers on the dorsal part and growth of the interdigital membrane. The central digit is longer.
12º	Fetus have a developed coelom cavity, an interdigital visible membrane and even more feathers are present in the dorsal and caudal regions.
13º	Fetus' beak contains a mandible with half the length of the maxilla and the differentiated phalanges. It is well observed the opening of the external auditory meatus as well as the differences in the phalanges.
14º	Fetus eyelids cover half of the eyeball with a nictant membrane. The beak contains a mandibular half-length maxillary process, grooves, and pigmentation on the free edge of the dorsal region of the maxillary process.
15º	Fetus' eyelids an almond-shaped opening. The beak is more pigmented, with deeper and more discernible grooves, presence of nostrils external auditory meatus system. Greater amount of feathers throughout the body.
16º	Albumen fully absorbed, full feathering, nipples, nails firmer, thoracic and pelvic limbs complete. The quail is ready to be born.

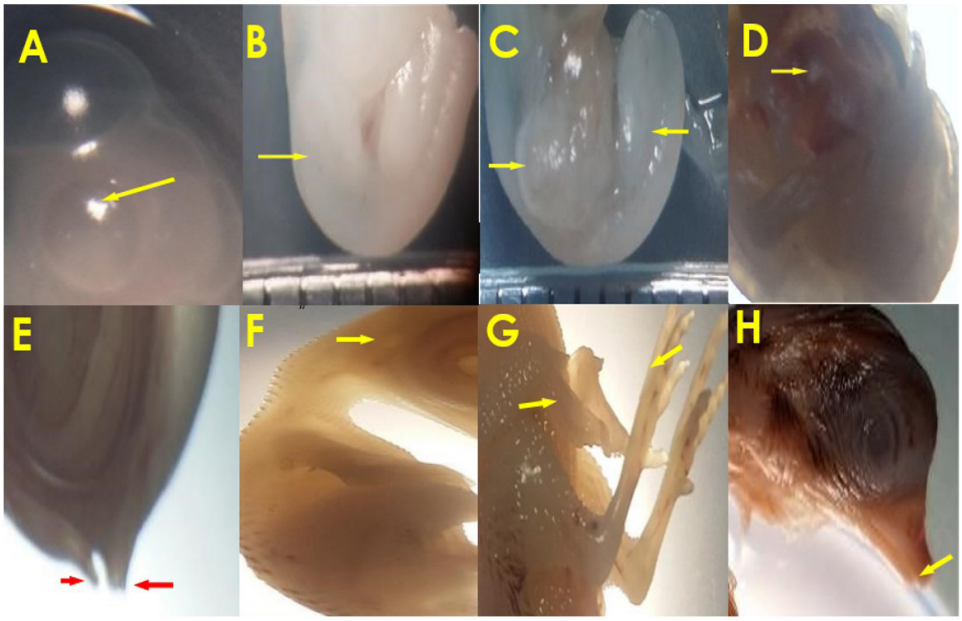

Figure 3. Highlighted external morphological areas.
Note: 5th day - eye opacity (arrow) (A); 6th day - "C" format and somites presence (arrow) (B); 7th day - thoracic and pelvic limb buds (arrows) (C); 11th day 9th day - visible internal organs. Heart (arrow) (D); 11th beak and mandibular process evident (arrow) (E); 12th day - visible auditory meatus (arrow) (F); 14th day - wings and legs defined (arrow) (G); 16th day - egg tooth defined (arrow) (H).

in the dorsal area and a clearly visible beak, and the beak and egg tooth became longer and more curved down. Stretching of the neck was noticeable at this stage, and the heart and liver were visible because the skin was transparent (Figure 2E). After 10 days of incubation (Figure 2D-2F), the eyes were large and very homogeneous and pigmented, with eyelids covering half of the eyeball in the presence of the nictating membrane; feathers were observed in the dorsal region, and the appearance of the digital rays and digits began to differentiate on the pelvic limbs. On day 11 of incubation, fetuses showed a more developed eyelid, appearance of the first follicles in the dorsal and posterior part of the forelimbs (Figure 2G), growth of the interdigital membrane, and the central digit farther away. The beak contained a mandible (Figure 3E) that was half the length of the jaw and the first observation of the auditory meatus. Pigmentation was observed in fetuses after 12 days of incubation in the dorsal region and some hair follicles in the pelvic region (Figure 3H), the auditory meatus was already visible (Figure 3F), and the interdigital membrane showed differences in phalanges. The celoma was well developed, and the cloaca was differentiated. After 13 days of incubation, fetuses showed greater distinction between the digits of the pelvic limb and an increasing presence of feather follicles around the cloaca and on the head (Figure 2I). Fetuses incubated for 14 days presented with all anatomical structures, each increasingly covered by feathers. The eyelid covered more than half of the eye, and the opening of the external auditory system was observed at this stage (Figure 2J). The beak contained a half-length mandible and was more pigmented, with the auditory meatus having more feathers at the edges. The claws were clearly visible, and the feet covered by papillae were observed (Figure 3H). The phalanges of the wings and feet, as well as grooves and pigmentation in the free edge of the dorsal region of the maxillary process, were also observed. The claws were observed at 14 days and the phalanges of the wings and feet were completely formed (Figure 3G). After 15 days of incubation, fetuses had eyelids with an almond-shaped opening. At this stage, the beak was more pigmented, with more deep and discernible furrows, the presence of nostrils, and the external auditory meatus was deeper, with feathery follicles at the edges. The development of the ribs was visible through the skin, which remained thin; nevertheless, at 15 days, the feather follicles were scattered throughout the body, and some included pigmentation (Figure 2K). Hair follicles were longer at 16 days, with three colors: dark brown, light brown, and beige (Figure 2L). Dark brown predominated on the back of the embryo and light brown predominated in the ventral region. The eyelids were closed, forming a slit downwards at the lateral side and the eyeball was less pronounced because they were covered with feathers. An opaque region was observed at the lateral side of the upper and lower jaws and at the location of the egg (Figure 3H). The external auditory meatus was rounded and encircled downwards; the sphere and the more keratinized claws were ready for hatching (Figure 2L).

Embryos weighed 0.0069 g at day 5 and 4.7863 g at day 16 (Figure 4), and the mean CRL was 0.368 cm and 3.657 cm, respectively (Figure 5). Weight and CRL increased by 0.434 g and 2.593 cm per day of incubation, respectively. The correlation between length and weight during incubation is shown in Figure 6.

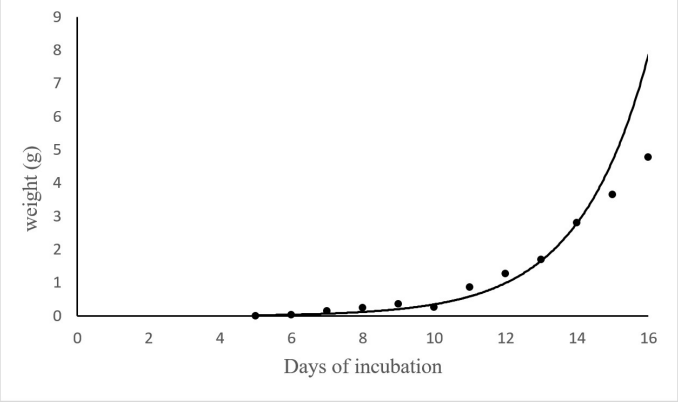

Figure 4. Quail embryos weight (g) from 5 until 16 days of incubation. Points show data and line represents the growth curve.

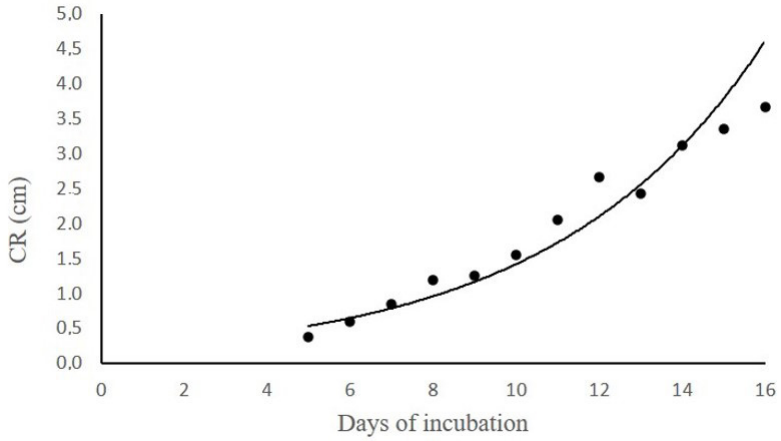

Figure 5. Quail embryos CR (cm) from 5 until 16 days of incubation. Points show data and line representes the growth curve.

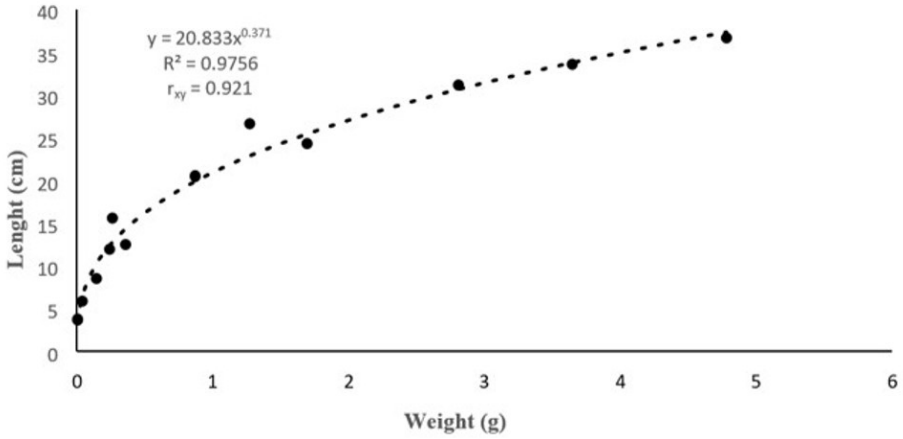

Figure 6. Correlation between length and weight during incubation days.

Discussion

The common morphological characteristics observed between 5 and 6 d of incubation in the present study were similar to those observed after 5-7 d in greater rhea (Almeida et al., 2015). Maximum "C" flexion in quail embryos occurred between 6 and 7 days, which is similar to the findings in 5-6-day rhea (Almeida et al., 2015) and 8-day ostrich embryos (Gefen & Ar, 2001).

The structure of the eyelid can be observed after 5 days of incubation in quail (Ainsworth et al., 2010; Ramteke et al., 2013), at 8 days in rhea (Almeida et al., 2015) and only at 14 days in ostrich (Gefen & Ar, 2001). In chick embryos, eyelids have been observed at 5.5 days (Hamburger & Hamilton, 1992).

In the present study, the jaw in the Japanese quail only appeared at 9 days, which 3 days later compared with that previously observed in quail (Ainsworth et al., 2010; Ramteke et al., 2013), and the 5.5 days in chicken (Hamburger & Hamilton, 1992), but shorter than the 11 days in rhea (Almeida et al., 2015).

The egg tooth and the nictitating membrane in the present study appeared at 9 and 10 days of incubation, respectively, which is different than the 6 days previously observed in quail (Ramteke et al., 2013) 10 days in rhea, (Almeida et al., 2015) and 14 days in ostrich (Gefen & Ar, 2001). The first signs of follicles occurred at 12 days; conversely, it appeared at 6 days in a previous study on quail (Ramteke et al., 2013), at 12 days in rhea (Almeida et al., 2015), 6.5 days in chicken, (Hamburger & Hamilton, 1992), and 16 days in ostrich (Gefen & Ar, 2001).

On day 13, feathers were observed over the entire body of the quail, which was also observed in the work on the rhea (Almeida et al., 2015). The morphological evolution of the eyelid in previous research occurred on days 14, 15, and 16 in quail, which was earlier than in another study (Ramteke et al., 2013); in rhea, this occurred between 14 and 18 days (de Almeida et al., 2015); and between 8 and 12 days in chicken (Hamburger & Hamilton, 1992).

The auditory meatus was observed at day 8 of incubation, in contrast to day 12 in rhea (Almeida et al., 2015) and day 5.5 in chicken (Hamburger & Hamilton, 1992).

The forelimb morphology in the quail embryos was similar to that in rhea (Almeida et al., 2015) and chicken embryos at day 4 (Hamburger & Hamilton, 1992) Pelvic limbs had differentiated digit growth at day 8, which is different from other studies in Japanese quail that showed the same development at day 7.5 day of incubation (Ainsworth et al., 2010) and at day 6 (Ramteke et al., 2013) in quail and day 5.5 in chicken (Hamburger & Hamilton, 1992), day 9 in rhea (Almeida et al., 2015), and day 16 in ostrich (Gefen & Ar, 2001).

In previous studies in Japanese quail, keratinization was observed in the first week of development, which was similar to the findings in rhea (Almeida et al., 2015) and chicken (Hamburger & Hamilton, 1992).

Closed eyelids forming slits were observed at day 14 of incubation; they were observed at day 13 in chickens (Hamburger & Hamilton, 1992), at day 21 in rhea (Almeida et al., 2015) and at day 26 in ostrich (Gefen & Ar, 2001).

Differences were observed between the morphological characteristics of quail embryos and fetuses compared with those in rhea and other bird species at the corresponding developmental stages. This may be due to the different methodologies used for incubation of each species. Morphological characterization of embryos and fetuses at different incubation ages was performed using morphological and external morphometric analyses.

In the present study, there was a strong correlation between the observed data and the day of incubation. An absence of correlation between chronological age and structure is often associated with individual egg size, differences in genetics and breeds, seasonal differences in embryo viability and vigor, in addition to incubation factors and incubator size (Hamburger & Hamilton, 1992). Moreover, the temperature difference and duration of incubation jointly influence morphogenesis (Dias & Müller, 1998).

Conclusions

In conclusion, after 16 days of incubation in a homemade incubator, the embryos presented all the phases of the development cycle, with appropriate development of weight and height based on the days of incubation without anomaly or external interference. These findings confirm that the homemade incubator does not alter the embryonic development of quail embryos and provides a good solution to increase the income of communities in the Brazilian Amazon region.

Ethics statement

The quail embryos study has been approved at the Animal Use Ethics Committee of the Universidade Federal do Oeste do Pará protocol number 10012/2017.

Financial support

None.

Conflict of interests

ACM, GCS, RTO, TSF, AALB and ASLS – No conflict of interest.

Authors' contributions

ACM – Advisor of the research group; contributed to the development of the experiment and the construction of the article; review and submission. GCS – Contributed to the construction of the incubator; monitoring and intellectual contribution in the preparation of the manuscript. RTO – Contributed to the construction of the incubator; opened and photographed all the

embryos; described all the particularities of the embryos in each phase. TSF – Built and adapted the incubator for quails; evaluated temperature and humidity indices to verify the viability of the embryos for hatching. ÂALB – Contributed to the construction of the incubator; helped to the experimental phase; helped in the preparation of the manuscript. ASLS – Intellectual contribution in the preparation of the manuscript.

Availability of complementary results

Ag Data Commons (ADC) (http://data.nal.usda.gov); AgBase (http://agbase.arizona.edu); Mendeley (http://data.mendeley.com); Global Biodiversity Information Facility – GBIF (http://www.gbif.org).

The study was carried out at Laboratório de Morfofisiologia Animal do Instituto de Biodiversidade e Florestas da Universidade Federal do Oeste do Pará, Santarém, PA, Brazil.

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