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Observations on the embryonic development of domestic meat-type guinea fowl (*Numida meleagris*)

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ABSTRACT

Guinea fowl breeding for meat production is widespread across Europe and the USA. For hatcheries to achieve their output potentials, they need in-depth knowledge about incubation techniques and guinea fowl embryonic development. The aim of this study was to provide updated quantitative data on the developing meat-type strain embryo and to describe its embryonic growth pattern in terms of embryonic weight modelled using Gompertz and logistic functions. Eggs from a 56-week-old genetically controlled flock (Galor S.A.S., Amboise, France) were individually weighed and incubated according to good hatchery practices. 10 embryos were randomly removed every 12 h through to hour 192 of incubation, and thereafter every 24 h. Incubation traits, blastoderm diameter, vitelline circulation diameter, and embryo body weight were recorded, and the mean daily wet embryo-specific mass was calculated. During each session, photographic documentation of the embryos was also obtained, including a general view of the egg content as well as the isolated embryonic body. Embryonic growth curves were estimated using Gompertz and logistic functions, and their parameters are given. High fertility (96%) and hatchability (81%) rates were observed, and the mean keet weight was 32 g at hatch. The accuracy of the curve fit was high for both models. The curves' inflection points occurred on days 21 and 23 for the logistic and Gompertz models, respectively, demonstrating an embryonic growth pattern typical of a precocial bird species. A photographic chart of the *in-ovo* chronological development of guinea fowl is provided.

HIGHLIGHTS

- The study follows the incubation and embryonic development of meat-type guinea fowl (*Numida meleagris*).
- Daily photographic images and graphical growth models of body weight document the birds' embryonic development.
- The results provide effective practical help for hatchery practices by enabling the determination of embryo age.

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Body weight; embryo development; growth curves; guinea fowl

Introduction

Interest in guinea fowl (*Numida meleagris*) farming for meat production is significant in several EU countries, such as France, Italy, and Belgium, as well as in the USA; and the commercialisation of this species has gained momentum over recent decades (Baeza et al. 2001; Nahashon et al. 2006a). In Africa, guinea fowl rearing is of high socio-cultural value, representing the second source of poultry meat and eggs after the chicken (Bernacki et al. 2013), and thus constitutes a valuable source of income (Moreki and Radikara 2013). Due to their low production costs and resistance to common poultry diseases, guinea fowl are also used as an alternative poultry species in free-range systems in Asia and Latin America (Yamak et al. 2018; Araújo et al. 2019; Krunt et al. 2021).

In order to make the guinea fowl productive system successful, hatcheries need to maintain high output performances and provide first quality guinea fowl keets. High output performances can be achieved with in-depth knowledge of modern incubation techniques

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and embryogenesis. Embryonic development is affected by several endogenous and exogenous factors, including the quality and quantity of nutrients contained in the egg, bird genotype, and incubation conditions (King'Ori 2011, Khalil et al. 2016, Nasri et al. 2020). Many studies have been published on the embryonic development and staging of domestic fowl in general (Hamburger and Hamilton 1951; Mun and Kosin 1960; Kaltofen 1971; Sellier et al. 2006; Ainsworth et al. 2010; Tong et al. 2013; Li et al. 2019), but few investigations have specifically been conducted on guinea fowl embryonic development (Ancel et al. 1995; Sellier et al. 2006; Araújo et al. 2019) and incubation techniques (Ancel et al. 1994a, 1994b).

Knowledge of the post-hatch growth pattern of an avian species and a corresponding prediction curve are essential tools for determining the potential growth capacity of selected birds raised under optimum rearing conditions, and for quantifying improvements in growth capacity obtained via genetic improvement programmes (Anthony et al. 1991; Nahashon et al. 2006a, 2006b; Castillo et al. 2020).

Little is known about the embryo staging of guinea fowl, and a developmental description of this species is lacking. These data are fundamental in hatchery practices for the evaluation of incubation technology output and the correct management of breeders. While the utility of embryo staging and development charts for poultry technicians is well recognised, embryonic growth models could also prove to be relevant in genetic improvement programmes as well as for hatchery practices,

The aim of the present study was to provide updated quantitative embryonic data and photographic evidence of the day-by-day development of meat-type guinea fowl in order to increase the current knowledge of this species' biology. As well as assess the embryonic growth patterns by means of predictive Gompertz and logistic models.

Materials and methods

The experiment was approved by the Ethics Committee of the University of Pisa (Italy) (Ref: OPBA_32/2021). A total of 550 hatching eggs laid by an artificially inseminated meat-type guinea fowl parent stock (Galor S.A.S, Amboise, France) were purchased from an industrial Italian breeding farm (Cairo s.r.l. Società Agricola, Santa Maria di Zevio, Verona, Italy); the flock was 56 weeks old. All eggs were delivered to our laboratory (Poultry farm 'Podere le Querciole', Department of Veterinary Science, University of Pisa) approximately 3 days post-oviposition and stored for 24 h in an 18 °C storage room at 75% relative humidity (RH). Eggs were labelled, individually weighed (Sartorius scale BL-150-S, readability 0.001 g, Sartorius AG, Göttingen, Germany), randomly divided into two batches and submitted to disinfection with a 0.02% quaternary ammonium compound solution. After a 6-hour pre-warming period at 25 °C and 75% RH, the two batches were set in a single-stage incubator (37.5 ± 0.01 °C and 57% RH) (model I9 Victoria Incubator, Victoria s.r.l., Pavia, Italy). On day 21 of incubation, the eggs were transferred to the hatchery (36.3 ± 0.1 °C and 82% RH) (model H4 Victoria Hatcher, Victoria s.r.l., Pavia, Italy).

The first batch of eggs was used to evaluate the incubation traits. On the 7th day of incubation, eggs were candled, and clear eggs and those containing dead embryos were removed and examined in order to assess true fertility (TF) and early mortality (eEM) rates. At the end of day 21 of incubation, the eggs were weighed to determine egg weight loss during the incubation period (ewl-IP), candled, and those with evidence of a living embryo were transferred into hatching baskets for hatchability (H) assessment. All eggs containing non-viable embryos at day 21 and all the unhatched eggs at the end of the incubation process were recorded to assess the late mortality (lEM) rate. TF, eEM, lEM, and H were calculated as follows:

$$\text{TF}\ (\%) = (\text{number fertile eggs}/\text{number of set eggs}) \times 100 \quad (1)$$

$$\text{eEM and lEM}\ (\%) = (\text{number of dead embryos}/\text{number of fertile eggs}) \times 100 \quad (2)$$

$$\text{H}\ (\%) = (\text{number of hatched keets}/\text{number of fertile eggs}) \times 100 \quad (3)$$

Total incubation duration was calculated as the time between setting and hatching, and the hatched keets were individually weighed to assess keet body weight (kBW). The keet yield on its fresh egg weight (keW) was obtained according to the following formula:

$$\text{keW}\ (\%) = (\text{kBW}/\text{fresh egg weight}) \times 100 \quad (4)$$

The second batch of eggs was used for the assessment of embryonic development: ten fertile eggs were randomly removed every 12 h, from hour 12 through to hour 192 of incubation, and thereafter every 24 h. Immediately after their removal from the incubator, eggs were weighed on the same Sartorius scale BL-150-S and prepared for embryonic evaluation according to the method described by Kaltofen (1971).

From hour 12 through to hour 108 of incubation, the blastoderm diameter (mm) and the apparent diameter of the vitelline circulation (mm) were measured using a calliper. From hour 120 of incubation, the embryos, isolated from the egg contents, were individually weighed to obtain the wet embryo body weight (weBW, g), and the specific mass of the wet embryo (spM) was determined. Starting from the beginning of the absorption phase of the *vitellum* in the abdomen, the embryo weight was determined following removal of the yolk-sac. The spM was calculated as:

$$\text{spM } (\%) = (\text{weBW}/\text{ fresh egg weight}) \times 100 \quad (5)$$

Photographs of the evaluated embryos were taken following partial removal of the shell and the testaceous membranes to obtain a general view of the egg content and the isolated embryonic body.

To estimate the expected body weight (BW) at a specific age, Gompertz and logistic 3-parameter growth curves (Bates and Watts 1988) were fitted to the weBW data collected, and the models obtained were used to assess the growth patterns of the pearl grey guinea fowl embryos.

The following equation describes the Gompertz growth model:

$$\text{Wt} = \theta 1 \exp\ [-\exp\ (\theta 2 - \theta 3 * x)] \quad (6)$$

The following equation describes the logistic growth model:

$$\text{Wt} = \theta 1/(1 + \theta 2 \exp(-\theta 3 * x)) \quad (7)$$

where Wt is the BW of an embryo at *t* time, and *x* is the age in days of the embryo.

The statistical analyses were performed using the program JMP 9.0.1 (SAS Institute Inc., 2009, Cary, NC), and the model's goodness-of-fit was assessed using R^2_{adj}.

Results and discussion

The mean egg weight was 49.97 ± 2.42 g. A lower mean egg weight (41.2 g) was reported by Kouame et al. (2019) for eggs obtained from breeders of the same French meat-type strain aged 32 weeks, while Ancel and Girard (1992) reported similar values to ours (48.9 g during the first laying cycle, 32–68 weeks of age). For meat-type Essor guinea fowl line (Hubbard SAS) aged 52 weeks, Nowaczewski et al. (2008) reported slightly higher egg weights (55.3 g) compared with those evaluated in our study. Lower egg weights are commonly observed in other guinea fowl domestic lines: in Polish guinea fowl, the mean egg weight is reported to be around 41.0 g (Nowaczewski et al. 2008; Bernacki et al. 2013); in Brazilian guinea fowl it is 37.6 g (Araújo et al. 2019); and in four Botswanan varieties egg weights are reported to range from 36.0–44.8 g (Kgwatalala et al. 2013); the lightest eggs are reported for the Nigerian strain (33.2 g) (Oso et al. 2020). The great variability observed in guinea fowl egg weight could be related to different selection goals for the studied strains.

Table 1. Domestic meat-type guinea fowl incubation traits.

Traits	Values
TF	96.40
eEM	4.56
lEM	14.11
tEM	18.67
H	81.33
ewl-IP, g	4.21 ± 0.67
ewl-IP, %	8.40 ± 1.28

Abbreviations. TF: true fertility; eEM: early embryonic mortality (0–7 days of incubation); lEM: late embryonic mortality (8–26 days of incubation); tEM: total embryonic mortality (0–26 days of incubation); H: hatchability on fertile eggs; ewl-IP: egg weight loss during the incubation period (0–21 days of incubation).

The incubation traits are reported in Table 1. The high fertility rate recorded in the present study (96%) is slightly higher than the value reported by Bernacki et al. (2013) in naturally mated grey polish guinea fowls (92%). The same authors observed a lower fertility rate in the white guinea fowl variety (85%) compared with the grey one. Similarly, Oso et al. (2020), Yamak et al. (2016), and Khairunnesa et al. (2016) observed lower fertility rates in naturally mated guinea fowl (56%, 76%, and 80%, respectively). As demonstrated by Mohan et al. (2016), lower fertility rates are observed in naturally mated guinea fowl flocks compared with artificially inseminated ones. The result from the present study supports this finding.

The embryo mortality rates observed in this study, at early as well as at late stages of development, were similar to those observed by Kouame et al. (2019) in the same meat-type strain (5.7% and 12.6%, respectively). The overall embryonic mortality rate in our study was 18.7%. Similar findings have been observed in polish grey guinea fowl (17.9%), whereas a slightly higher mortality rate was reported for the white polish variety (21.8%) (Bernacki et al. 2013). Hatching took place at 636 ± 6 h of incubation, and the hatchability of the used fertile eggs was very similar to that recorded for the same strain by other authors (Ancel et al. 1994a; Kouame et al. 2019). Instead, great variation in hatchability results can be observed when different guinea fowl lines are considered (Bernacki et al.

Figure 1. Photographic chart of the *in-ovo* chronological development of the domestic meat-type guinea fowl.

2013; Khairunnesa et al. 2016; Mohan et al. 2016; Yamak et al. 2018; Oso et al. 2020).

Egg weight loss at day 21 of incubation was approximately 8.4% (Table 1). Kouame et al. (2019), using the same Galor strain but a longer incubation period (0–23) and different incubation parameters (37.7 °C and 55% RH), reported an average egg weight loss equal to 11.2%, comparable to that reported by Oso et al. (2020), who recorded a value of 11.5% in a Nigerian strain over a 24-day incubation period. The reasons for the variability in the observed incubation traits between different authors and strains remain to be determined but are likely to involve factors related to the management of the breeders, the quality of the eggs produced by different populations, and factors related to the applied incubation environments and protocols.

Table 2. Domestic meat-type guinea fowl wet embryo body weight (weBW, g) from day 5 to day 26 of incubation (mean daily values ± SD).

Days of incubation	weBW, g
5	0.073 ± 0.015
6	0.168 ± 0.009
7	0.304 ± 0.078
8	0.552 ± 0.073
9	0.954 ± 0.071
10	1.211 ± 0.066
11	1.742 ± 0.092
12	2.478 ± 0.124
13	3.211 ± 0.170
14	4.333 ± 0.185
15	5.846 ± 0.506
16	6.796 ± 0.460
17	8.598 ± 0.527
18	11.290 ± 0.490
19	13.087 ± 0.921
20	15.529 ± 1.089
21	18.325 ± 1.061
22	22.119 ± 0.561
23	23.210 ± 0.569
24	25.476 ± 1.452
25	28.149 ± 1.575
26	30.213 ± 0.440

Upon hatching, keets showed a mean body weight of 31.67 ± 1.49 g, resulting in a 65.73 ± 2.24% mean yield on the fresh egg weight. A similar keet weight in the same strain was reported by Baeza et al. (2001). In that study, a mean keet weight of 31.4 g was reported for the standard fast-growing Galor line, and 32.8 g for the slow-growing Galor line. Lower keet weights have been reported for an American strain of guinea fowl (25.5 g; Nahashon et al. 2006a) and a Turkish line (26.7 g, Yamak et al. 2018).

The mean daily blastoderm diameter and mean vitelline circulation diameter from day 0.5 to day 4.5 of incubation are reported in Figure 1. The main external macroscopic changes in the developing embryo are also presented. The mean weBW and spM values of the developing embryos from day 5 to day 26 of incubation are presented in Table 2 and Figure 1, respectively. The same parameters were reported by Ancel et al. (1995), who found slightly smaller diameters and lower spM values compared with the present study. Since the same genotype was used in both studies, the differences recorded could be the result of several years of genetic improvements in this meat-type guinea fowl strain.

The modelled growth curves described by the Gompertz and logistic three-parameter equations are represented in Figure 2, and the estimated theta values of each equation are reported in Table 3. The R^2_{adj} value was 0.996 and 0.995 for the logistic model and Gompertz model, respectively; these values demonstrate a good fit of both models, meaning that either

Table 3. Calculated theta values for the Gompertz and logistic equations for domestic meat-type guinea fowl developing embryos.

Theta values	Gompertz equation	Logistic equation
$\theta 1$	57.507	35.814
$\theta 2$	2.580	490.728
$\theta 3$	0.116	−0.298

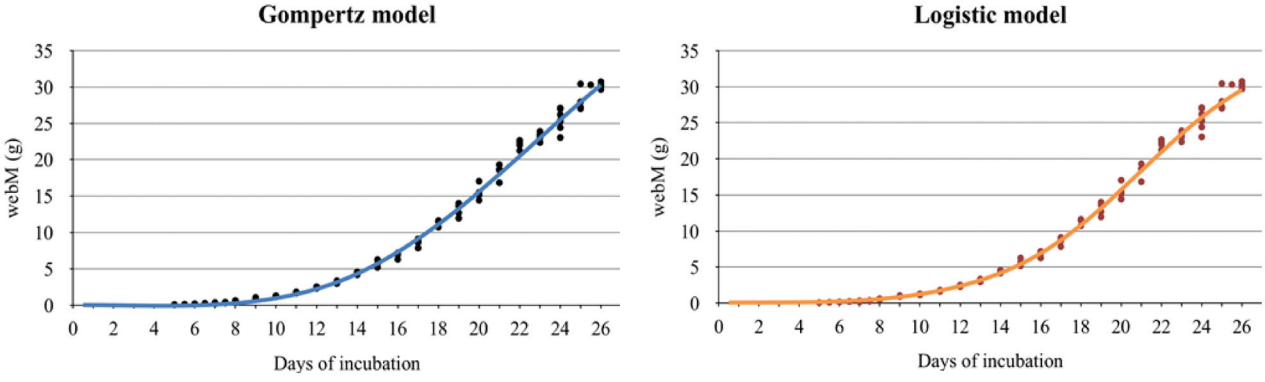

Figure 2. Growth curves of domestic meat-type guinea fowl embryos as predicted by Gompertz and Logistic models.

equation can be used to accurately estimate guinea fowl embryo body weight at different ages of development.

The inflection points for the two models occurred on days 21 and 23 of incubation when the embryos achieved their maximum daily weight gain: 2.66 g and 2.46 g for the logistic and Gompertz models, respectively. Forty years ago, Vleck et al. (1980) demonstrated using the Gompertz model that the maximum absolute growth rate in bird embryos occurs before hatching in precocial species, but after hatching in altricial species. Starck and Ricklefs (1998) defined guinea fowl as a precocial bird based on hatchling developmental maturation patterns, parent-chick interactions, and chick-environment interactions. Our findings provide further support to this designation on the basis of guinea fowl embryonic growth patterns.

Conclusions

The results presented here represent a further step in the comprehension and evaluation of domestic guinea fowl embryonic growth by means of mathematical growth models. The photographic chart and the updated data constitute an effective practical tool for determining the exact age of meat-type guinea fowl embryos during their *in-ovo* development. Furthermore, the present data highlight that the selective progress affected, not only the post-hatch performances but also the pre-hatch life of this domestic guinea fowl line. Determining the age of embryos from opened eggs is an important hatchery practice. It constitutes a fundamental process in the evaluation of dead embryos, which can help to identify any major problems that may be occurring during incubation. Such problems may be related to hatchery procedures, or even to the management of the breeder birds themselves. The ability to identify the age of embryos may also be useful during the conduction of experimental tests. Although the difficult task, embryo aging can be achieved with good accuracy through the use of correct models and quantitative data.

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Disclosure statement

No potential conflicts of interest are reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, A.S., upon reasonable request.

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