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# Incubation parameters and post-hatch sexing methods in Wyandotte hens

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**ABSTRACT**

The preservation of breeds and their characterization allows the maintenance of a genomic diversity that can be crucial to face new challenges of sustainability in livestock farming. The Wyandotte breeder has a dual purpose, meat and eggs, besides having docility and rusticity characteristics, favoring its breeding in small and large scales. In this study, the influence of egg storage time on incubation performance and criteria for determining the sex of post-hatch birds were evaluated. Three egg storage periods were adopted: short (0-2 days), intermediate (3-5 days) and long (6-8 days). The total weight loss between storage and incubation was higher for eggs that were stored longer (6-8 days) compared to the other evaluated times. The hatchability rate was better for the eggs that had been stored for less time. The embryonic mortality of eggs with longer storage time (6-8 days), showed a higher number of eggs with early mortality and stillborn. At hatching, it was possible to differentiate the chicks by the abdominal plumage coloration, with the males characterized by yellow coloration from the crop to the cloaca region. The yellow coloration was observed in the female only in the cloacal region. The prolonged storage time negatively affects weight loss of eggs from the Wyandotte breeders, as well as incubation parameters; and the post-hatch sexing can be performed by evaluating the plumage coloration of the chocks ventral area on hatch day.

## 1. Introduction

The increase in extreme weather events attributed to climate change has been a factor that motivates the adoption or improvement of the sustainability of livestock production, with poultry and eggs being attributed to an 8% share in the anthropogenic greenhouse gas production (Gerber et. al, 2013). On the other hand, the consumption of poultry meat and eggs has been associated with high consumer interest in poultry welfare (Vanhonacker and Verbeke, 2009) inducing the adoption of alternative production systems. Mixed crop-livestock production systems can improve food production, environmental preservation and achieve high animal welfare standards (Rocchi et al. 2019).

The Wyandotte breed was cataloged by the American Standard of Perfection[1] in 1883, originally from North America, of dual purpose, meat and egg production, and also breed as an ornamental bird, because it has an exuberant plumage. In relation to weight, they are considered heavy birds, with the males weighing an average of 3.9 kg and the females 3 kg. The eggs of this breed are brown eggshell and weigh an average of 55 grams with annual laying that can reach 220 eggs in the first cycle (Gerhardt, 2015; Oliveira, 2014; Ekarius, 2007). The Wyandotte breed is rustic and has a good ability to adapt to hot or cold climates, and has a docility characteristic, which facilitates its breeding in confinement or on small farms (Gerhardt, 2015).

Studies determining productive and reproductive parameters of this breed are scarce, and mostly date back to the 1930s and 1940s, which report issues of choice of ingredients employed in the feed and the relationship of these with egg laying, studies on preference in the choice of the breed by small breeders and on the color characteristics of its eggs (Lee, 1921; Elkhorabi et al., 2014; Kimball, 1955).

One of the key points of the adoption of a laying hen in larger production scale is their propagation, requiring the determination of the ideal period for egg storage, condition that may be related to the lineage or breed and/or the different weight categories (light, semi-heavy or heavy), age of the breeder and even between geographic regions (Calil, 2013). During egg storage and incubation there is a considerable weight loss, which is additive, and the function of weight loss at the time of incubation is important for the formation of the air chamber. Under long storage conditions, the weight loss can be explained by the reduction in the water vapor pressure deficit across the shell (Meijerhof, 2013).

Another fundamental factor for the propagation of hens, especially breeders, and for egg production is the early determination of sex. For the Wyandotte breed, this possibility of post-hatch sexing is not described in the literature. This management was one of the great technical advances of poultry breeding, because it allowed to separate chicks by sex in production batches, i.e., creating males separated from females, enabling a better uniformity of the flock (Cotta, 2012).

The aim of this study was to evaluate the results of incubation parameters of eggs from Wyandotte hens as a function of different storage periods and the sexing of one-day-old birds.

1 The American Standard of Perfection is the official breed standard for poultry in North America, classifies and describes the standard physical appearance, color, and temperament for all recognized breeds of birds, including chickens, ducks, geese, and chickens. The current edition was published in 2015.

## 2. Material and Methods

The study was conducted with 219 eggs (average weight 53 g), from 55-week-old Wyandotte breeder hens, reared in the Experimental Didactic Poultry Laboratory (LADEX) of the Canguiri Experimental Farm of the Federal University of Paraná in a cage-free system, with floor recovered by wood shave, perches (20 cm per poultry) and nests (one per four hens) with one male for every seven females. The eggs were daily collected, immediately stored for batch formation and then incubated. After hatching, 100 birds were evaluated to determine the best criterion to be adopted for the segregation assuming the hypotheses that external differences in plumage coloration can be related to the sexual expression (male x female) in this breed.

### 2.1 Egg collection and storage

Eggs were collected daily in the breeding house, previously selected and classified into clean eggs, dirty eggs, litter eggs and nest eggs, and dirty eggs with blood stains, feces and yolk were discarded. Only the nest clean eggs were then cleaned with a non-abrasive sponge, removing only light soiling such as fluff and shavings. Then, the eggs were stored in the storage room at a temperature of 17°C and a relative humidity of 60%, as recommended in the Cobb Hatchery Management Guide (2020).
Subsequently, the eggs were evaluated by ovoscopy, and eggs with cracks, splits and opaque eggs were discarded. Then, the first weighing (weight at the beginning of storage) and identification (numbering of the eggs for control). The eggs were placed in incubation trays, separated by days of storage, which ranged from zero to eight days, divided into three periods:

- 1 – Storage: 0-2 days (n=73 eggs).
- 2 – Storage: 3-5 days (n=73 eggs).
- 3 – Storage: 6-8 days (n=73 eggs).

### 2.2 Pre-incubation management

The temperature of the storage room was adjusted at +2°C every 45 minutes for 5 hours, so that there was a gradual temperature increase, thus avoiding the formation of droplets and condensation of eggs. The eggs were weighed again (day of incubation weight) to determine the percent weight loss between storage and incubation.

### 2.3 Incubation eggs

The egg trays were placed inside the experimental single-stage incubator, Avicomave model, which was previously adjusted to a temperature of 37.5°C and relative humidity (RH) of 65%. The eggs were randomly distributed in 3 trays allocated in the middle of the incubator. Each tray had space for the storage of 96 eggs, in which 73 spaces were used (number of eggs in the three periods evaluated).
Daily the physical parameters were checked and noted: temperature, RH, ventilation and the turning process, this being programmed for a 45° turning every hour.

### 2.4 Handling of egg transfer from setter to hatcher

The hatcher was previously prepared with the temperature set at 36.9°C and RH of 85%. The transfer of the eggs occurred at 18th day of incubation (438 hours), in which the eggs were weighed for the third time (weight at transfer) to calculate the percentage of weight loss between the periods of storage and transfer to the hatcher.
To select the eggs considered fertile, all incubated eggs were scanned, and the translucent eggs were sent for embryodiagnosis and the eggs considered fertile (opaque in the scans) were placed in the hatcher trays, keeping the separation of the three storage periods. The physical parameters of the hatcher were checked and recorded daily: temperature and RH.

### 2.5 Birth and sexing of the chicks

At the time of withdrawal (~510 hours) from the hatcher, the number of hatching eggshells were initially recorded to determine the start of the hatch window and the chicks were kept in the hatcher for a maximum period of 24 hours after hatching. Subsequently, the birds were weighed and separated by sex (males and females). To determine the sexing criteria, 100 chicks were evaluated in the hatchery and inspected for their exterior, considering external differences in: wing fheater size, visual characteristics of the cloaca; body plumage and fheater cover and coloration differences between anatomical topographic parts covered with different shades. Differences in sexing the above variables were adopted as criteria for the chick's segregation at day old.

### 2.6 Embryodiagnosis

The eggs that did not hatch were sent for embryodiagnosis. Embryodiagnosis was performed to determine the number of infertile eggs, contaminated eggs and in case of fertile eggs the embryonic mortality stages, the data collected were used to calculate incubation performance indices. The eggs were individually opened in a glass plate for visual evaluation and confirmation as infertile and/or fertile eggs, the latter being evaluated regarding the period of embryonic mortality. Embryonic mortality observed in fertile eggs was classified into the following phases, which were adapted from Plano and Matte (2008):

- M1 – Early mortality: 1th to 7th days.
- M2 – Intermediate mortality: 8th to 18th days.
- M3 – Late mortality: 19th to 21th days.
- M4 – Stillborn.

## 2.7 Calculation of incubation performance indices

The formulas described by Rosa and Avila (2000) were used for the calculations of % hatching, hatchability, and fertility:

$$\text{(A)}\ Hatching = \frac{total\ chicks\ hatched}{total\ number\ of\ eggs\ incubated} \times 100$$
$$\text{(B)}\ Hatchability = \frac{total\ chicks\ hatched}{total\ fertile\ eggs} \times 100 \qquad (1)$$
$$\text{(C)}\ Fertility = \frac{total\ fertile\ eggs}{total\ number\ of\ eggs\ incubated} \times 100$$

## 2.8 Equations for determining the weight loss of eggs

(a) Loss of weight during storage (A) = average egg weight in storage – egg weight in incubation.
(b) Weight loss in incubation (I) = average egg weight in incubation – average egg weight at transfer. (2)
(c) Total weight loss = average egg weight in storage – average egg weight on transfer.

## 2.9 Accommodation and confirmation of segregated sexed chicks

The chicks were initially housed in two boxes in a brooding house equipped with a heating system using lamps, wood shaving and the feed and water supply were ad libitum, separated according to the adopted sexing criteria, and evaluated again at 18th weeks of age.

## 2.10 Experimental design and Statistical analysis

The experimental design was completely randomized, with 3 treatments for storage time with 73 eggs for each treatment. The response variable to the storage time was the egg weight loss during the storage, during the incubation and during the total time between the storage to the end of incubation period (18th day of incubation).
The data from the three weighings were analyzed using the Statistix 9.0 program and were subjected to analysis of variance (ANOVA) and the means were compared using the Tukey test. Considering that no reference parameters are available for Wyandotte´s hatched eggs, the incubation performance was presented by hatching, hatchability, fertility and embryonic mortality in percentage.

## 3. Results and discussion

The results obtained in the three egg weighings (storage, incubation and transfer from incubator to hatcher), were compared in relation to the three storage times, being the average weights in storage, incubation and transfer, respectively: 0-2 days – 53.8 ± 0.5, 53.8 ± 0.5, 47.4 ± 0.5; 3-5 days – 54.0 ± 0.4, 54.0 ± 0.4, 46.9 ± 0.4; and 6-8 days – 53.8 ± 0.4, 53.4 ± 0.4, 46.6 ± 0.4, which showed no significant statistical differences (P>0.05). There was a greater weight loss of eggs in grams and in percentage (P<0.001) for eggs with storage time between six and eight days, being greater weight loss compared to the other two periods that did not differ (Table 1).
The storage times did not influence (P>0.05) the egg weight in grams or in percentage during the incubation period (day zero) until transfer (18th day of incubation). Otherwise, cumulatively weight loss in grams and percent between the total period of storage to the transfer, was lower in the up to two days storage period, differing statistically from the longer period (6-8 days; P=0.016), both without differing from the intermediate (3-5 days) storage period. The weight loss of eggs during the storage period was minimal (<1g), but this loss added to the other periods, demonstrates that the 6-8 days storage period has an influence on the total weight loss.
In a study conducted with eggs from Dekalb breeders, the researchers observed that eggs from older breeders lost more weight, regardless of the storage period, which can be attributed to the lower shell thickness of these birds compared to those from younger breeders (Tanure, 2009). However, our results show that the weight loss of eggs from 55-week-old Wyandotte hens has a direct relationship with storage time.
For adequate embryonic development, the weight loss of the egg during the incubation period should be between 10 and 14% (Gonzáles et al., 2009). We observed that the lower weight loss of the eggs in our study demonstrated better hatchability indices, which were presented in Table 2, where the hatchability index result was higher at the 0-2 day storage time, as well as the hatchability.

| Storage time | Weight loss A[1] (g) | Weight loss I[2] (g) | Total weight loss[3] (g) | % Weight loss A[1] | % Weight loss I[2] | % Total weight loss[3] |
|---|---|---|---|---|---|---|
| 0-2 days | 0,0 ± 0,06[b] | 6,43 ± 0,23 | 6,43 ± 0,23[b] | 0 ± 0,1[b] | 11,9 ± 0,4 | 11,9 ± 0,4[a] |
| 3-5 days | 0,0 ± 0,05[b] | 7,06 ± 0,18 | 7,07 ± 0,18[ab] | 0 ± 0,1[b] | 13,1 ± 0,3 | 13,1 ± 03[ab] |
| 6-8 days | 0,45 ± 0,05[a] | 6,74 ± 0,18 | 7,19 ± 0,18[a] | 0,9 ± 0,1[a] | 12,7 ± 0,3 | 13,4 ± 0,3[b] |
| CV % | 225 | 23 | 23 | 223 | 23 | 22 |
| P-value | 0,000 | 0,091 | 0,282 | 0,000 | 0,090 | 0,016 |

[1]A = weight loss during the storage; [2]I = weight loss during incubation; [3]Total weight loss between storage at the transfer.
CV %: coefficient of variation in %.
[a,b,c]: Means with different letters in the same columns are significantly different *($P<0.05$)* by *Tukey* test.

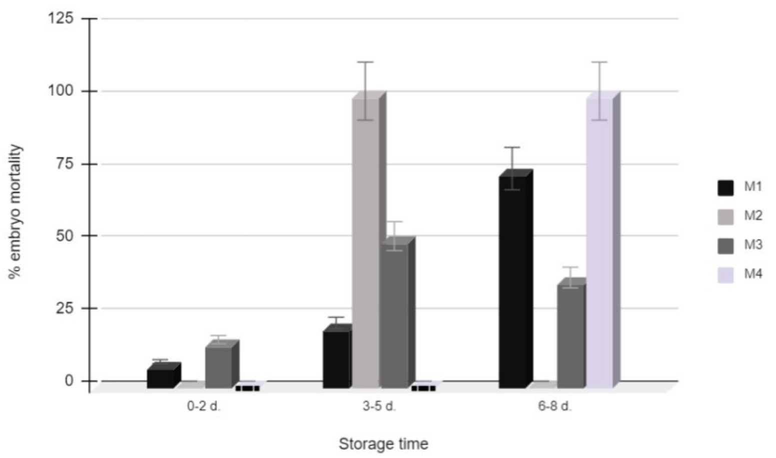
***Table 1** – Hatching eggs weight loss from Wyandotte hens (g) and in percentage at the beginning of storage, incubation and transfer, in relation to the different storage times.*

Tanure et al. (2009), report that the best hatchability indices are observed when the weight loss is around 12%, corroborating with our results, where we observed that the percentage of weight loss was 11.9% and the hatchability index 92.86% (respectively presented in Tables 1 and 2) for the period of lower storage of eggs.

| Storage time | N° of incubated eggs | N° of hatched eggs | Hatching (%) | Fertility (%) | Hatchability (%) |
|---|---|---|---|---|---|
| 0-2 days | 73 | 52 | 71,23 | 76,71 | 92,86 |
| 3-5 days | 73 | 37 | 50,68 | 65,75 | 77,08 |
| 6-8 days | 73 | 30 | 41,10 | 67,12 | 61,22 |

***Table 2** – Number of incubated and hatched eggs, calculated rates of hatchability, hatching and fertility indices in percentage, in relation to the different storage times.*

According to Decuypere and Micheles (1992), increasing the storage period of one day can reduce the incubation performance by 1% and increase the incubation time of these eggs by one hour. Mousa-Balabel and Saleem (2004) described that the hatchability index can be affected by the storage period and also that an increase in initial embryonic mortality and late mortality can be a result of this storage, which was observed in the evaluation of embryonic mortality in this study, performed by embryodiagnosis, as shown in Graph 1.




***Graph 1** – Embryo mortality at three different storage times (zero to 2; 3 to 5; and 6 to 8 days of storage).*

The embryonic mortality of eggs with longer storage time (6-8 days) had the highest number of eggs with early mortality (M1= 73.3%), and stillborn (M4= 100%). Followed by the intermediate storage time, which we observed the highest number of eggs with intermediate embryonic mortality (M2= 100%) and late embryonic mortality (M3= 50%).

The fertile egg storage temperature influences the embryonic mortality in its initial phase (Mahmud et al., 2011), and the storage temperature of eggs from heavy breeders should be below the physiological zero (20°C), because eggs stored above the physiological zero start their development disproportionately (earlier and/or later) leading to malformation and embryonic mortality (Muraroli and Mendes, 2003). Our study adopted a temperature of 17ºC, below the physiological zero to ensure the complete stop of embryonic development, as we opted for a longer storage period (8 days) to obtain a greater number of eggs for incubation.

The shortest egg storage time (0-2 days) showed the lowest number of eggs classified in embryodiagnosis in the different embryonic mortality periods (M1= 6.7%; M2= 0%; M3= 14.3%; M4= 0%).

The birds after hatching were weighed and evaluated following the three storage times, where we observed no statistically significant differences, with the mean weight in grams at 0-2 days being 35.30 ±0.43 ; 3-5 days being 35.29 ± 0.40; and at 6-8 days being 34.73 ± 0.45. The hatch window was from one to three days, which we did not observe statistical differences between storage times and hatch window (P>0.05), however, Reis et al. (1997), report that eggs arising from a longer storage period may present a longer incubation period, due to a possible delay in embryonic development.

To determine the criteria to be adopted for sexing, the chicks were evaluated in the hatchery for differences in wing fheater size, visual characteristics of the cloaca and coloration of the plumage at hatching. The last criterion was the one adopted for differentiation, where birds with yellow coloration from the crop to the cloaca were characterized as males (Figure 1a) and birds with only yellow cloaca were considered females (Figure 1b/ 1c).


***Figure 1*** *– External characteristic observed by different plumage coloration in one-day old chicks between crop and cloacal body area.*

The chicks were raised in a house, separated (males and females), and at 18th weeks of age we observed the growth of the comb and wattles in the males, confirming the correct sexing performed by the characterization described above. This characterization is important in the formation of new production flocks, making it possible to separate males and females at birth in order to apply the correct nutritional management for each sex guide selection programs.

Due to the possibility of storing eggs for the formation of incubation flocks, together with the possibility of sexing on the first day of age, it allows the Wyandotte breed to be used in multiplication and selection projects without major difficulties. It is still necessary the zootechnical characterization of the breed, growth curve, survey of reproductive indicators and verification of the possibility of forming sexable hybrids.

## 4. Conclusion

Prolonged storage time decreases the egg weight from parent hens of the Wyandotte breed, as well as the incubation indices of hatching and hatchability percentages. It's possible sexing day old Wyandotte chicks only by external inspection. The sexing can be observed through the difference in color of the abdominal area at birth, allowing the separate breeding of males and females.

**Background notes:** CEUA – UFPR CERTIFICATE: 007/2021

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