

## Article

# Functional Effects of Single-Stage vs. Multi-Stage Incubation Systems and Parental Flock Age on Embryonic Development, Oxidative Stress, and Performance of Male Broiler Chickens

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## Abstract

It is well established that both the age of the breeder hen and the type of incubator can influence the efficiency of the hatching process. However, there is a lack of information in the literature regarding the interaction between these two factors. This study evaluated the effects of incubator type (multi-stage vs. single-stage) and breeder hen age (35 and 61 weeks) on the hatching parameters, embryonic oxidative stress, performance, carcass yield, and meat quality of male broiler chickens. The embryo livers from the multi-stage incubator presented significantly higher NADP oxidase (NOX) values ( $p = 0.022$ ), indicating elevated oxidative stress. A significant interaction between breeder age and incubator type was observed for the thiol concentrations, with embryos from older hens incubated in the multi-stage system showing higher thiol levels compared to those from the single-stage system. Birds from these older breeders demonstrated increased breast yield, feed intake, and weight gain, without significant changes in feed conversion ratio. Additionally, the single-stage incubator was associated with reduced embryonic oxidative stress, lower egg weight loss during incubation, and improved early performance of chicks during the first week post-hatch. In conclusion, beyond the previously recognized benefits of single-stage incubation systems, our findings highlight their potential to mitigate oxidative stress in embryos, thereby enhancing early chick development.

**Keywords:** breast yield; embryonic mortality; NADP oxidase; single-stage incubator



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## 1. Introduction

Poultry production is one of the agribusiness sectors that has achieved continuous growth since it was conceived, making birds increasingly productive and specialized for their purpose [1]. In this scenario, Brazil stands out as the third-largest producer of chicken meat worldwide and the largest exporter of this commodity [2]. With an increasingly competitive market, the search for success in breeding these animals has mainly focused on the sanity, environment, and nutrition of birds, often disregarding problems arising from the breeder hens or egg hatching. The short life of the broiler can justify this, usually

slaughtered with 42 days, where genetics, air quality, and nutrition would have a greater observable impact. Despite this, there is a consensus that breeder hens of different ages influence the weight and quality of chicks [3–5], such as large egg embryos, which have a better development due to the greater amount of nutrients available [3,6].

Hatcheries play a crucial role in the production of one-day-old chicks, utilizing two types of commercial incubators. In the multi-stage (MS) system, eggs are added continuously, resulting in the presence of embryos at various developmental stages [7]. When incubated in MS machines, thermal energy generated by older embryos is transferred to younger ones, leading to an excessive rise in internal setter temperature. This uncontrolled thermal interaction can result in suboptimal incubation conditions and increased embryonic mortality [8].

On the other hand, according to [9], the single-stage (SS) machines are fully loaded with a single egg lot, thus allowing better control of temperature, humidity, and ventilation. As a result, all embryos are at the same developmental stage, which enables precise control of incubation parameters, tailored to the specific physiological needs of the embryos throughout the incubation process.

The success of the poultry production chain, which include hatcheries and breeder hens, can be seen through increased egg hatching, chick viability [9], the weight of animals, and good navel healing [10]. Few studies have addressed the age of the breeder hens and incubator type, although the age of the breeder hen is known to affect several variables related to the hatching and the quality of the chick, especially concerning variables related to the oxidative stress of the embryos and their consequences on performance and meat quality.

Thus, this study aimed to evaluate the effects of the type of incubator (multi-stage and single-stage) and the age of the breeder hen (35 and 61 weeks) on variables related to the hatching yield, embryo oxidative stress, performance, carcass and cut yields, and meat quality of male broilers raised up to 42 days of age.

## 2. Materials and Methods

The study was conducted in Chapecó, state of Santa Catarina, Brazil. The first phase, involving egg incubation, took place in a commercial hatchery where eggs from breeder hens aged 35 and 61 weeks—belonging to the agro-industrial cooperative Cooperativa Central Aurora Alimentos (Chapecó, Santa Catarina, Brazil)—were incubated. The second phase was carried out at the experimental poultry house of the Experimental Farm of the Universidade do Estado de Santa Catarina (UDESC). Following the rearing period, the birds were slaughtered in a commercial processing plant, and laboratory analyses were performed at the Laboratory of Animal Products Technology, Department of Animal Science, UDESC.

### 2.1. Experimental Design and Treatments

A completely randomized block design was adopted for the hatching trial, following a  $2 \times 2$  factorial arrangement (2 incubator types and 2 breeder hen ages), with eight replicates consisting of trays containing 86 eggs per machine. Three machines of each incubator type were used as blocks, and no significant block effect was observed for any of the incubation variables evaluated ( $p > 0.05$ ).

Fertile eggs from two Ross 308 (Aviagen) broiler breeder flocks of 35 and 61 weeks of age, with average weights of  $60 \pm 3.2$  g and  $72 \pm 4.5$  g, respectively, were produced on the same day and received the same care (collection, selection, and disinfection). The eggs were stored in the hatchery for four days at an average temperature of 20 °C and humidity of 75%, and then they were preheated (28 °C for 12 h) before hatching.

The single- and multi-stage incubators used were Casp (Model 125e) and Coopermaq multi-stage (Model INC 1290), respectively, with a capacity for 124,000 eggs each. The eggs were packed in trays with a capacity for 86 eggs, arranged in trolleys with 36 trays each. In the multi-stage machine, the eggs were placed weekly. The same number of eggs from each stage of incubation was placed within the setter and distributed across the incubation period.

In both incubator types, initial temperature was set at 99.3 °F and relative humidity (RH) at 58%. On day 18 of incubation (432 h), in ovo vaccination against Marek's disease was performed. Eggs were then transferred to a hatcher (CASP® 108 HR, Hatcher Plus Pte Ltd., Singapore), with a capacity of 19,264 eggs, set to maintain 98.6 °F temperature and 65% RH. The experiment was completed at 504 h of incubation, when hatched chicks were removed from the hatch baskets.

The experimental plots (eight trays with 86 eggs per repetition) were distributed evenly within each incubator, one in each cart, and always in the center. Percentages of infertile eggs; hatchability; hatching of fertile eggs; mortality from 0 to 4, 5 to 18, and 19 to 21 days; total embryonic mortality; and weight loss at hatching were evaluated. These percentage values were calculated in relation to the total number of eggs per tray (86 eggs), while the weight losses during hatching were calculated by the differences between the weight of the trays + eggs at the beginning and 18.5 days of hatching before their transfer to the hatchery.

For the performance evaluation, 420 one-day old male chickens from the incubation trial were distributed in a  $2 \times 2$  factorial arrangement (two incubator types and two breeder hen ages), with seven replicates consisting of 15 birds each.

## 2.2. Performance

The birds were housed in 2 m<sup>2</sup> pens with reused litter (three cycles of production) and equipped with tube feeders and nipple drinkers. Water and feed were provided ad libitum. Diets were formulated based on corn and soybean meal according to the nutritional requirements and food composition established by the Brazilian Tables for Poultry and Swine [11] and prepared in a horizontal mixer with a capacity of 150 kg. Feed intake, weight gain, feed conversion, and viability were evaluated in the periods of 1 to 7, 1 to 21, 1 to 35, and 1 to 42 days of age by weighing the feed and birds at the beginning and end of each breeding phase. Flock viability (%) was calculated by dividing the final number of live birds by the initial number housed at the beginning of the trial.

To quantify carcass and cut yields, at 42 days of age, two birds per pen were randomly chosen, weighed, and taken to slaughter, with an eight-hour fast and two-hour pre-slaughter rest. At the slaughterhouse, birds were weighed again, obtaining the slaughter weight, which was used as a reference to measure carcass yields (carcass weight/slaughter weight  $\times$  100) and cuts (chest, legs, wings, back, and abdominal fat), obtained through the relation of their weights with the weight of the respective carcass.

## 2.3. Biochemical Variables Related to Oxidative Stress

At 18.5 days of hatching, two eggs were separated per tray and broken, and the livers of the embryos were collected after verifying their deaths through vital signs. The samples were identified, placed in a cooler, and sent refrigerated to the laboratory of Veterinary Biochemistry, Universidade Federal de Santa Maria—UFSM—to analyze the reactive oxygen species (ROS), substances responsive to the thiobarbituric acid (TBARS), protein thiol (PT), and NADP oxidase (NOX), according to the methodologies described by [12–15], respectively.

## 2.4. Physical Chemical Analysis of the Meat

The breasts were deboned and the pectoralis major muscles were packed in plastic bags, identified, stored in thermal boxes, and sent to the laboratory for analysis after stabilizing from rigor mortis (five hours after slaughter).

The pH was measured in triplicate, in the cranial region of the muscle, using a digital pH meter (model 205, Testo, Campinas, SP, Brazil). Meat color was determined in the inner part of the Pectoralis major muscle, using a Minolta Chroma Meter model CR-400, which determined the parameters of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ).

Water-holding capacity (%) was determined using a sample of 2 g ( $\pm 0.15$ ) of meat from the Pectoralis major. These samples were placed between two filter papers and acrylic plates and received a pressure exerted by weight of 10.0 kg for five minutes. After this period, the samples were weighed once again to determine the water retention capacity, as described by [16].

Cooking loss was evaluated using the methodology proposed by [17], where breast meat samples were packed in plastic bags with initial weight identified and taken to a water bath for 30 min at 85 °C. At the end of this period, the samples were taken from the plastic bags for cooling and water disposal and weighed again for comparison, thus determining the percentage of cooking losses.

The shear force was measured using the same samples that underwent cooking loss, reduced in size with known measurements, and accommodated with the muscle fibers oriented perpendicularly to the WarnerBratzler blade coupled to the Texture Analyser TA-XT2i Texturometer, which measured the shear force, expressed in  $\text{kgf}/\text{cm}^2$  [18].

## 2.5. Statistical Analysis

All variables were tested for normality using the Shapiro–Wilk test. For animal mortality, which did not follow a normal distribution, the non-parametric Kruskal–Wallis test was applied ( $p < 0.05$ ). Subsequently, the data were analyzed using analysis of variance (ANOVA) through the General Linear Model (GLM) procedure in SAS software (Statistical Analysis System, version 9.0). When significant effects were detected, the means of the factorial treatment ( $2 \times 2$ ) were compared using the Fisher–Snedecor test ( $\alpha = 0.05$ ).

## 3. Results

### 3.1. Fertility, Hatching, and Biochemical Parameters

The eggs produced by the older breeder hens showed significantly higher percentages of infertile and embryonic mortalities from 5 to 18 and 19 to 21 days and for total mortality and weight loss in hatching. The rates of hatchability and hatching of fertile eggs were higher ( $p < 0.001$ ) in the eggs of young breeder hens (Table 1).

**Table 1.** Incubation parameters (%) of eggs from breeders of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

|        | Breeder Age<br>(Weeks—BA) |       |       | Incubator Type<br>(IT) |       |       | $p^{BA \times IT}$ | CV (%) |
|--------|---------------------------|-------|-------|------------------------|-------|-------|--------------------|--------|
|        | 35                        | 61    | $p$   | SS                     | MS    | $p$   |                    |        |
| IE     | 1.38                      | 13.02 | **    | 7.86                   | 6.32  | 0.211 | 0.132              | 46.25  |
| HAT    | 93.75                     | 79.34 | **    | 85.22                  | 87.87 | 0.075 | 0.080              | 4.24   |
| HFE    | 94.85                     | 90.73 | *     | 91.85                  | 93.73 | 0.078 | 0.188              | 3.10   |
| M4     | 2.63                      | 5.30  | **    | 4.92                   | 3.02  | *     | **                 | 36.25  |
| M5-18  | 0.67                      | 1.69  | 0.011 | 1.71                   | 0.65  | **    | 0.960              | 47.10  |
| M19-21 | 1.10                      | 3.17  | 0.014 | 2.11                   | 2.17  | 0.90  | 0.325              | 54.67  |
| TEM    | 5.14                      | 9.26  | **    | 8.14                   | 6.26  | 0.071 | 0.468              | 38.09  |
| HWL    | 12.09                     | 13.57 | *     | 11.96                  | 13.70 | *     | 0.129              | 2.72   |

\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; CV = coefficient of variation. IE: infertile eggs; HAT: hatchability; HFE: hatching of fertile eggs; M4: mortality from 0 to 4 days; M5-18: mortality from 5 to 18 days; M19-21: mortality from 19 to 21 days; TEM: total embryonic mortality; HWL: hatching weight loss.

There was a significant effect of the type of incubator (TI) with lower values for multi-stage in the analysis of embryonic mortality from 5 to 18 days and greater weight loss in hatching for the same incubator ( $p < 0.001$ ).

There was a significant interaction between the factors breeder age (BA) and type of incubator (TI) for the variable mortality from 0 to 4 days of hatching, observing higher mortality for the embryos of the 61-week breeder hens only in the single-stage incubator. There was also a significant effect of the type of incubator ( $p < 0.0001$ ), with higher mortality of the embryos of older birds hatched in the single-stage machine (Table 2).

**Table 2.** The interaction between the factors breeder age (BA) and incubator type (IT) for the variable percentage of mortality from 0 to 4 days of hatching.

| Incubator    | Breeder Age (Weeks) |      |             |
|--------------|---------------------|------|-------------|
|              | 35                  | 61   |             |
| Single-Stage | 2.24                | 7.60 | $p < 0.001$ |
| Multi-Stage  | 3.03                | 3.02 | $p = 1.00$  |
|              | $p = 0.680$         |      | $p < 0.001$ |

Rows: Mean values for each incubator type in the different breeder hen ages. Columns: Mean values for each breeder age, considering both incubator types.

There was no effect of the age of the breeder hen on the biochemical variables evaluated ( $p > 0.05$ ). However, there was a higher NOX value ( $p = 0.022$ ) in the samples of embryo livers from the multi-stage incubator (Table 3).

**Table 3.** Hepatic biochemical variables of embryos from breeder hens of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

|       | Breeder Age (Weeks—BA) |        |       | Incubator Type (IT) |        | $p^{BA \times IT}$ | CV (%) |
|-------|------------------------|--------|-------|---------------------|--------|--------------------|--------|
|       | 35                     | 61     | $p$   | SS                  | MS     | $p$                |        |
| ROS   | 18,117                 | 14,611 | 0.112 | 17,560              | 15,168 | 0.256              | 35.10  |
| TBARS | 51.25                  | 41.86  | 0.088 | 47.51               | 45.60  | 0.690              | 31.00  |
| TP    | 0.054                  | 0.053  | 0.750 | 0.049               | 0.058  | 0.038              | 22.0   |
| NOX   | 7.23                   | 6.81   | 0.520 | 6.20                | 7.85   | 0.015              | 25.98  |

CV = coefficient of variation. ROS: reactive oxygen species (U DCFH/mg protein); TBARS: thiobarbituric acid-reactive substances ( $\mu\text{mol MDA/mg protein}$ ); PT: protein thiols ( $\text{mmol SH/mg protein}$ ); NOX: NADP oxidase ( $\mu\text{mol NOX/mg protein}$ ).

There was a significant interaction between the BA and TI factors for the thiols variable (Table 4), where embryos from older hens in the multi-stage incubator presented higher values compared to single-stage ones.

**Table 4.** The interaction between the factors breeder age (BA) and incubator type (IT) for the biochemical variable protein thiols ( $\text{mmol SH/mg protein}$ ).

| Incubator    | Breeder Age (Weeks) |       |             |
|--------------|---------------------|-------|-------------|
|              | 35                  | 61    |             |
| Single-Stage | 0.055               | 0.044 | $p = 0.31$  |
| Multi-Stage  | 0.054               | 0.062 | $p = 0.49$  |
|              | $p = 0.99$          |       | $p = 0.028$ |

Rows: Mean values for each incubator type in the different breeder hen ages. Columns: Mean values for each breeder age, considering both incubator types.

### 3.2. Performance

Birds from breeder hens of 61 weeks of age showed significantly higher initial weight, feed intake, and weight gain values in all breeding stages (Table 5). The age of the breeder hen did not influence feed conversion ( $p > 0.05$ ) in any of the periods evaluated. Feed intake and weight gain were lower in multi-stage incubators only from 1 to 7 days.

**Table 5.** Performance variables of broiler chickens from breeders of different ages and hatched in single-stage (SS) and multi-stage (MS) machines in the different breeding periods evaluated.

|            | Breeder Age<br>(Weeks—BA) |       |       | Incubator Type<br>(IT) |       | $p^{BA \times IT}$ | CV (%) |
|------------|---------------------------|-------|-------|------------------------|-------|--------------------|--------|
|            | 35                        | 61    | $p$   | SS                     | MS    | $p$                |        |
| 1 to 7 d.  |                           |       |       |                        |       |                    |        |
| IW         | 0.042                     | 0.050 | *     | 0.046                  | 0.046 | 0.941              | 1.95   |
| FI         | 0.167                     | 0.178 | 0.056 | 0.181                  | 0.169 | **                 | 8.65   |
| WG         | 0.143                     | 0.156 | *     | 0.155                  | 0.143 | *                  | 5.52   |
| FC         | 1.17                      | 1.14  | 0.14  | 1.17                   | 1.18  | 0.141              | 7.14   |
| FV         | 100                       | 100   | ---   | 100                    | 100   | ---                | ---    |
| 1 to 21 d. |                           |       |       |                        |       |                    |        |
| FI         | 1.222                     | 1.300 | **    | 1.469                  | 1.177 | 0.587              | 4.14   |
| WG         | 0.978                     | 1.057 | *     | 0.985                  | 0.957 | 0.077              | 3.80   |
| FC         | 1.250                     | 1.230 | 0.124 | 1.240                  | 1.230 | 0.75               | 2.74   |
| FV         | 98.33                     | 97.77 | 0.672 | 98.88                  | 97.22 | 0.211              | 3.22   |
| 1 to 35 d. |                           |       |       |                        |       |                    |        |
| FI         | 3.560                     | 3.734 | **    | 3.645                  | 3.649 | 0.979              | 3.77   |
| WG         | 2.552                     | 2.704 | **    | 2.624                  | 2.631 | 0.869              | 4.03   |
| FC         | 1.395                     | 1.381 | 0.291 | 1.389                  | 1.387 | 0.863              | 2.07   |
| FV         | 96.66                     | 97.22 | 0.764 | 97.22                  | 96.66 | 0.761              | 4.57   |
| 1 to 42 d. |                           |       |       |                        |       |                    |        |
| FI         | 5.029                     | 5.237 | **    | 5.141                  | 5.121 | 0.721              | 3.83   |
| WG         | 3.311                     | 3.464 | **    | 3.414                  | 3.360 | 0.329              | 3.94   |
| FC         | 1.519                     | 1.512 | 0.641 | 1.506                  | 1.524 | 0.269              | 2.20   |
| FV         | 96.11                     | 96.11 | 0.999 | 96.11                  | 96.11 | 1.00               | 5.73   |

\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; CV = coefficient of variation. IW: initial weight (kg); FI: feed intake (Kg); WG: weight gain (kg); FC: feed conversion (kg/Kg); FV: flock viability (%).

Higher breast yield ( $p = 0.013$ ) and a tendency for lower leg yield ( $p = 0.054$ ) were observed in the carcasses of birds from the 61-week breeder hens (Table 6). The other cuts were not influenced. The type of incubator did not significantly affect the yield of carcass and cuts.

**Table 6.** Carcass and cuts yields (%) and abdominal fat (A.F.) of birds from breeder hens of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

|         | Breeder Age<br>(Weeks—BA) |       |       | Incubator Type<br>(IT) |       | $p^{BA \times IT}$ | CV (%) |
|---------|---------------------------|-------|-------|------------------------|-------|--------------------|--------|
|         | 35                        | 61    | $p$   | SS                     | MS    | $p$                |        |
| Carcass | 75.42                     | 76.19 | 0.148 | 75.88                  | 75.72 | 0.759              | 1.64   |
| Breast  | 40.30                     | 42.10 | 0.013 | 41.28                  | 41.11 | 0.792              | 3.93   |
| Leg     | 30.18                     | 28.77 | 0.054 | 29.30                  | 29.65 | 0.442              | 3.76   |
| Wings   | 10.26                     | 9.94  | 0.360 | 10.18                  | 10.01 | 0.611              | 8.12   |
| Back    | 18.30                     | 17.88 | 0.449 | 17.89                  | 18.28 | 0.479              | 7.34   |
| A. F.   | 1.07                      | 0.84  | 0.141 | 0.91                   | 0.99  | 0.579              | 37.79  |

CV = coefficient of variation.

The age of the breeder hen did not significantly affect the evaluated meat quality parameters (Table 7). On the other hand, the type of incubator influenced ( $p < 0.0001$ ) the variable water retention capacity, with a higher value found in the meat of the birds hatched in a multi-stage machine.

**Table 7.** Meat quality related-variables of broiler chickens from breeder hens of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

|     | Breeder Age<br>(Weeks—BA) |       |       | Incubator Type<br>(IT) |       |       | $p^{BA \times IT}$ | CV (%) |
|-----|---------------------------|-------|-------|------------------------|-------|-------|--------------------|--------|
|     | 35                        | 61    | $p$   | SS                     | MS    | $p$   |                    |        |
| pH  | 5.68                      | 5.71  | 0.441 | 5.69                   | 5.70  | 0.941 | 0.159              | 1.91   |
| L   | 54.14                     | 54.26 | 0.866 | 54.38                  | 54.02 | 0.586 | 0.744              | 3.01   |
| a * | −0.64                     | −0.93 | 0.503 | −0.79                  | −0.77 | 0.533 | 0.781              | 73.04  |
| b * | 9.86                      | 9.50  | 0.593 | 9.27                   | 10.09 | 0.238 | 0.241              | 16.97  |
| WHC | 70.69                     | 70.88 | 0.833 | 69.43                  | 72.14 | **    | 0.821              | 3.07   |
| CL  | 17.51                     | 15.78 | 0.408 | 17.05                  | 16.24 | 0.695 | 0.382              | 30.08  |
| SF  | 2.081                     | 2.174 | 0.683 | 2.025                  | 2.234 | 0.365 | 0.607              | 26.81  |

\*\*  $p < 0.01$ ; CV = coefficient of variation. L: lightness; a \*: redness; b \*: yellowness; WHC: water-holding capacity (%); CL: cooking losses (%); SF: shear force (kgf/cm<sup>2</sup>).

#### 4. Discussion

The higher fertility rate observed in eggs from 35-week-old breeder hens is related to the fact that males are practically at the beginning of their reproductive life. There is a higher cover index and sperm quality during this phase [19]. The company's farms that supplied the eggs use the so-called "spiking" for males and "intra-spiking" management in batches of older breeder hens to minimize this drop in fertility. However, the results show that the drop in fertility is still significant in older birds. Therefore, it can be said that the hatchability and the hatching of fertile eggs tend to be higher in the eggs of young birds if there are higher fertility rates, if no deviations occur during the hatching process, as was observed in the present study.

Embryonic mortalities at different hatching ages and weight loss at hatching were lower in young breeder hens due to the better internal and external quality of the eggs since the quality of the albumen, yolk, and shell change with the age of the hen and influence mortality [20]. The volume of albumen decreases with the advancement of the productive period, even with the increase in the size of the egg. Therefore, its functions of supplying water, minerals, and amino acids are compromised, affecting the contribution necessary for the embryo [21]. On the other hand, the yolk increases its volume due to the smaller interval between ovulations [22]. The shell tends to increase in area but not in number of pores, which allows a large loss of water by the embryo despite improving the oxygen supply [23], favoring egg weight loss during hatching [24,25].

Weight loss related to the multi-stage incubator was observed; in addition to this, weight loss was also associated with the age of the breeder hen. This is due to the moisture imbalance in the openings, the egg tray exchange, and the consequent heat generation of the metabolism of different ages within the same space [26]. Therefore, the incubator has lower levels in these openings in which the humidity is controlled when receiving the external air from the hatching room, where a temperature exchange and low relative humidity inside the incubator occurs.

The observed result of the interaction between type of incubator and age of the breeder hen, where older hens had higher mortality in the single-stage system, contradicts the work conducted by [27], who demonstrated the need for this type of incubator for eggs that have larger pores and therefore have more chances of dehydration. However, this research

showed no evidence to support the result found in the interaction between the age of the breeder and the type of incubator.

The higher concentration of NOX observed in samples from multi-stage incubators may be related to the higher temperature oscillation within this equipment, generating thermal stress in embryos [28]. This increase in temperature generates positive feedback for the increase in blood insulin, a physiological response to reduce heat production that previously used lipid molecules to maintain body temperature and now transports glucose to be oxidized in the bloodstream [29]. Furthermore, the increase in NOX may also be linked to the release of inflammatory cytokines due to the caloric stress they undergo during hatching [30], apoptosis of immune cells, and the lower activity of natural immune system killer (NK) cells [31]. Embryos from older hens incubated in multi-stage systems showed increased protein thiol levels (Table 4), suggesting a more effective antioxidant response to the elevated NADPH oxidase (NOX) activity observed in these embryos.

The higher values observed for FI and WG of birds from older breeder hens are explained by the fact that older hens produce larger eggs and, consequently, heavier birds at slaughter. However, it is important to note that the FC was not altered, as was also observed by [32]. According to [3], as the age of the hen increases, feed intake and weight gain of the broilers also increase.

In this study, birds from older breeder hens did not show higher initial mortality. According to [33], this is related to the better initial performance of animals in weight gain, feed intake, and food conversion.

Higher FI and WG were observed during the first seven days of hatching in the single-stage machine. This is due to the biosecurity provided by this type of equipment, which has a lower contamination index and allows the animal to have an adequate development [34], including obtaining higher rates of navel healing and leg quality [32]. These characteristics are essential for chickens housed in reused beds, as is the case of the present study, in addition to factors such as better temperature distribution and humidity control, which can cause stress to the embryo and impair the initial performance of animals. This difference in performance occurred only in the first days of breeding since the birds hatched in multi-stage incubators develop immunity and acquire resistance to microorganisms. Therefore, no significant differences in FI and WG occur after this initial period.

The birds from the older breeder hens had higher breast yield and greater weight gain. Heavier slaughtered birds have more meat deposited in the carcass. However, there is usually no increase in the percentage of cut yield, as observed in the reports of [35].

The meat of the birds hatched in a single-stage incubator showed lower water retention capacity; that is, they lost more liquid as a result of the pressure received. This result is contrary to expectations since these birds theoretically had less oxidative stress during hatching. According to [36], oxidation or oxidative stress decreases water solubility and binding capacity, especially when it affects proteins, thus promoting a greater loss of water in the meat.

## 5. Conclusions

This study demonstrates that the interaction between breeder age and incubator type significantly influences embryonic development. Embryos from older hens showed higher mortality between 0 and 4 days of incubation, but only when incubated in single-stage systems. In contrast, embryos from older breeders incubated in multi-stage systems exhibited increased protein thiol levels, suggesting a more effective antioxidant response to the elevated NADPH oxidase (NOX) activity observed in these embryos.

When analyzed independently, single-stage incubators were associated with lower NOX activity in embryos, reduced egg weight loss during incubation, and improved chick

performance during the first week of life. Although breeder age negatively affected fertility and general incubation parameters, chicks from older hens demonstrated superior post-hatch performance and higher breast yield, indicating potential compensatory advantages in later developmental stages.

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## Abbreviations

The following abbreviations are used in this manuscript:

|       |                                         |
|-------|-----------------------------------------|
| BA    | Breeder age                             |
| IT    | Incubator type                          |
| TBARS | Thiobarbituric acid-reactive substances |
| SS    | Single-stage                            |
| MS    | Multi-stage                             |
| ROS   | Reactive oxygen species                 |
| TP    | Protein thiols                          |
| NOX   | NADP oxidase                            |

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