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Article

Incubator Type and the Broiler Breeder Hen Age Affects Embryo Oxidative Stress, Hatching Yield, Performance and Meat Quality of Broiler Chickens

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Abstract

It is already known that both the age of the breeder hen and the type of incubator can influence the efficiency of the hatching process. However, the literature lacks information on the interaction of these factors. We evaluated the effects of incubator machine type (multi-stage and single-stage) and breeder hen age (35 and 61 weeks) on hatching parameters, oxidative stress of the embryo, performance, carcass yield and meat quality of the male broiler chickens. The embryo livers from the multi-stage incubator presented a higher NOX value ($p=0.022$). We observed a significant interaction between the breeder age and type of incubator factors for the thiols variable, where embryos from older multi-stage hens presented higher values compared to single-stage ones. The chickens from these birds had higher breast yield, feed intake, and weight gain without changing the feed conversion. The single-stage incubator provided lower oxidative stress to the embryos, lower egg weight losses during hatching and better performance of the birds in the first week of life. The chickens from the older breeders presented higher breast yield, feed intake and weight gain. In addition to the known advantages of single-stage incubators, this work showed that they cause less oxidative stress to embryos.

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

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 in its 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 was mainly focused on the sanity, ambiance 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, ambiance, and nutrition would have a greater observable impact. Despite this, there is a consensus that breeder hens of different ages influence the quality of chicks [3,4,5], such as large egg embryos, which have a better development due to the greater amount of nutrients available [6].

Hatcheries also play a key role in the production of one-day chicks, provided with two types of commercial hatching systems. The multi-stage receives eggs continuously, thus harboring embryos at different stages of development [7], and the single-stage, where incubators are stocked with eggs from the same batch, thus allowing better control of temperature, humidity, and ventilation [8].

The success of these sectors, 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 addressed the age of the breeder hens and hatching systems, 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 zootechnical 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 hatching yield, embryo oxidative stress, performance, carcass and cuts yields and meat quality of male broilers raised up to 42 days of age.

2. Materials and Methods

The study was conducted in the municipality of Chapecó, SC, Brazil. The first stage (egg hatching) was conducted in a commercial hatchery, where eggs were hatched from breeder hens of 35 and 61 weeks of age, belonging to an agroindustry of the region. The second stage of the experiment was carried out in the experimental poultry house of the Experimental Farm of the Universidade do Estado de Santa Catarina - UDESC. The birds were slaughtered in a commercial slaughterhouse, and laboratory analyses were carried out in the Laboratory of Animal Products Technology of the UDESC, Department of Animal Science.

2.1. Experimental design and treatments

A completely randomized design was used for both stages of the study, in a 2 X 2 factorial arrangement (2 incubator types and 2 breeder hen ages). Eight replicates (trays with 86 eggs each) were used for the variables related to hatching and seven for the performance test, with 15 birds per pen.

Fertile eggs from two Ross broiler breeder flocks with 35 and 61 weeks of age 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%, then they were preheated (28°C for 12 hours) before hatching.

The single and multi-stage incubators used were Coopermaq multi-stage (Model INC 1290) and Casp (Model 125e), respectively, with a capacity for 124,000 eggs each. The eggs were packed in trays with a capacity for 86 eggs, arranged in carts with 36 trays each. In both incubators, 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), with a capacity of 19,264 eggs, set to maintain 98.6 °F temperature and 65 % RH. The experiment was completed at 504 hours of incubation, when hatched chicks were removed from the hatch baskets.

The experimental plots (eight trays with 86 eggs per treatment) were distributed evenly within each incubator, one in each cart, 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.

2.2. Performance

Four hundred and twenty-one-day male chicks from the first stage of the study were divided into four treatments with seven replicates of 15 birds each. The birds were housed in 2 m² pens with reused litter (three cycles of production) and equipped with tube feeders and nipple drinkers. Water and feed were provided ad libitum and diets 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.

To quantify carcass and cuts yield, 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, 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 (Testo, model 205). 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 luminosity (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 minutes at 85 °C. At the end of this period, the samples were taken from the plastic bags for cooling and water disposal, 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 kgf/cm² [18].

2.5. Statistical analysis

All variables were subjected to normality testing (Shapiro–Wilk). For animal mortality, that did not show normal distribution, the non-parametric Kruskal–Wallis test was used (5% or $P < 0.05$). Subsequently, the data were subjected to ANOVA and F test, in which a difference between groups was considered when $P < 0.05$.

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. Mean values obtained for the percentages of infertile eggs (IE), hatchability, (HAT), hatching of fertile eggs (HFE), mortality from 0 to 4 days (M4), mortality from 5 to 18 days (M5-18), mortality from 19 to 21 days (M19-21), total embryonic mortality (TEM) and hatching weight loss (HWL) of eggs from breeders of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

	Breeder Age (Week - BA)			Incubator Type (IT)			$P_{BA \times IT}$	CV (%)
	35	61	P	SS	MS	P		
IE	1.19	13.02	*	7.86	6.32	0.249	0.115	52.87
HAT	93.75	79.34	**	85.22	87.87	0.080	0.079	4.74
HFE	94.85	90.73	**	91.85	93.73	0.078	0.198	3.13
M4	2.63	5.30	**	4.92	3.02	**	**	39.44
M5-18	0.67	1.69	0.014	1.71	0.65	0.010	0.960	49.40
M19-21	1.10	3.17	0.014	2.11	2.17	0.92	0.343	56.66
TEM.	5.14	9.26	**	8.14	6.26	0.078	0.197	40.37
HWL	12.09	13.57	**	11.96	13.70	**	0.158	2.97

* $P<0.001$; ** $P<0.01$; CV = coefficient of variation.

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 eggs of older birds hatched in the single-stage machine (Table 2).

Table 2. Breakdown of 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	$P = 1.00$
	$P = 0.740$	$P<0.001$	

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. Mean values obtained for the hepatic biochemical variables reactive oxygen species (ROS, U DCFH/mg protein), thiobarbituric acid-reactive substances (TBARS, η mol MDA/mg protein), protein thiols (PT, mmol SH/mg protein), and NADP Oxidase (NOX, μ mol NOX/mg protein) of embryos from breeding hens of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

	Breeder Age (Week - BA)			Incubator Type (IT)			$P_{BA \times IT}$	CV (%)
	35	61	P	SS	MS	P		
ROS	18117	14611	0.122	17560	15168	0.286	0.077	37.14
TBARS	51.25	41.86	0.091	47.51	45.60	0.724	0.148	31.71
TP	0.054	0.053	0.808	0.049	0.058	0.042	0.034	22.23
NOX	7.23	6.81	0.541	6.20	7.85	0.022	0.197	27.43

CV = coefficient of variation.

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

Table 4. Breakdown of the interaction between the factors breeder age (BA) and incubator type (IT) for the biochemical variable thiols (mmol SH/mg protein).

Incubator	Breeder Age (Weeks)		
	35	61	
Single Stage	0.055	0.044	P= 0.33
Multi-stage	0.054	0.062	P= 0.49
	P= 0.99	P=0.030	

3.2. Performance

Birds from breeder hens with 61 weeks 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. Mean values obtained for the variables initial weight in kg (IW), feed intake in Kg (FI), weight gain in kg (WG), feed conversion (FC) and flock viability (FV,%) of birds coming from different age breeders and hatched in single-stage (SS) and multi-stage (MS) machines in the different breeding periods evaluated.

	Breeder Age (Week - BA)			Incubator Type (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	0.161	1.95
FI	0.167	0.178	0.056	0.181	0.169	**	0.423	8.65
WG	0.143	0.156	*	0.155	0.143	*	0.703	5.52
FC	1.17	1.14	0.14	1.17	1.18	0.141	0.231	7.14
FV	100	100	---	100	100	---	---	---
1 to 21 d.								
FI	1.222	1.300	**	1.469	1.177	0.587	0.417	4.14
WG	0.978	1.057	*	0.985	0.957	0.077	0.181	3.80
FC	1.250	1.230	0.124	1.240	1.230	0.75	0.311	2.74
FV	98.33	97.77	0.672	98.88	97.22	0.211	0.671	3.22
1 to 35 d.								
FI	3.560	3.734	**	3.645	3.649	0.979	0.384	3.77
WG	2.552	2.704	**	2.624	2.631	0.869	0.236	4.03
FC	1.395	1.381	0.291	1.389	1.387	0.863	0.477	2.07
FV	96.66	97.22	0.764	97.22	96.66	0.761	0.140	4.57
1 to 42 d.								

FI	5.029	5.237	**	5.141	5.121	0.721	0.857	3.83
WG	3.311	3.464	**	3.414	3.360	0.329	0.863	3.94
FC	1.519	1.512	0.641	1.506	1.524	0.269	0.582	2.20
FV	96.11	96.11	0.999	96.11	96.11	1.00	0.999	5.73

* P<0.001; ** P<0.01; CV = coefficient of variation.

Higher breast yield (P=0.013) and a tendency to 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. Values obtained for 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 (Week - BA)			Incubator Type (IT)			P _{BA x IT}	CV (%)
	35	61	P	SS	MS	P		
Carcass	75.42	76.19	0.148	75.88	75.72	0.759	0.428	1.64
Breast	40.30	42.10	0.013	41.28	41.11	0.792	0.276	3.93
Leg	30.18	28.77	0.054	29.30	29.65	0.442	0.743	3.76
Wings	10.26	9.94	0.360	10.18	10.01	0.611	0.529	8.12
Back	18.30	17.88	0.449	17.89	18.28	0.479	0.432	7.34
A. F.	1.07	0.84	0.141	0.91	0.99	0.579	0.654	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. Mean values obtained for the variables pH, luminosity (L), redness (a*), yellowness (b*), water holding capacity (WHC,%), cooking losses (CL,%) and shear force (SF, kgf/cm2) of poultry breast meat from breeder hens of different ages and hatched in single-stage (SS) and multi-stage (MS) machines.

	Breeder Age (Week - BA)			Incubator Type (IT)			P _{BA x 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

CV = coefficient of variation.

4. Discussion

The higher fertility rate observed in eggs from breeder hens with 35 weeks is related to the fact that males are practically at the beginning of 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 "spinking" 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

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 function 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 will allow 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 relative to 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 finds 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 will occur. 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, contradicts the work conducted by [27], who demonstrates 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 age x 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 underwent during hatching [30], apoptosis of immune cells, and the lower activity of natural immune system killer (NK) cells [31].

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-stages 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 showed lower water retention capacity, that is, lost more liquid with the pressure received. This result is contrary to the expected since these birds theoretically had less oxidative stress during hatching. According to [36], oxidation or oxidative stress decreases water's solubility and binding capacity, especially when it affects proteins, thus promoting a greater loss of water in the meat.

5. Conclusions

The age of the breeder hen negatively affected the fertility rate, embryonic mortality, and egg weight losses during hatching. However, the chickens from these birds had higher breast yield, feed intake, and weight gain, without changing the feed conversion.

Single-stage incubators provided lower oxidative stress to embryos, lower egg weight losses during hatching, and better performance of birds in the first week of life.

Supplementary Materials: None

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Conflicts of Interest: The authors declare no conflicts of interest.

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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