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Original Article

Evaluation of the Relationship Between the Breeder's Age, Breed and the Incubator Types on Quality and Animal Welfare in the Hatchery

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ABSTRACT

About 33.3% of chicken life is conditioned by the result of embryonic development. Therefore, understanding and improving the early phase of the broiler's life is critical to guarantee their well-being. Thus, the objective of this research was to evaluate the relationship between the breeder's age, breed and the incubator types on quality and animal welfare in the hatchery. The experimental design was completely randomized in a 2 x 2 x 3 factorial arrangement (incubator, breed and age), with 12 treatments and 6 replicates each. The eggs derived of Cobb 500 and Ross's breeders at the beginning of the laying period, middle age and at the end of the laying period. It is concluded that the size of the eggs is related to the breeder's age and breed, which influences directly the number of bled eggs. In addition, well-being in the hatchery can be affected by decreased hatching and contamination that is higher in older breeders. Cobb eggs also need more attention because they are larger, therefore, they have a higher risk of breakage and they are more sensitive to the low concentration of CO_2 at the hatcher.

INTRODUCTION

About 33.3% of chicken life is conditioned by the result of embryonic development. Therefore, understanding and improving the early phase of the broiler's life is critical to guarantee their well-being.

Preferably, between 17 days and 6 hours up to 18 days and 5 hours of incubation, in egg vaccination may be performed (Fernandes *et al.*, 2016), during the moment of transference from the incubators to the hatchers. This type of vaccination has several advantages, among them: the reduction of the stressful response associated with the procedures of post-hatch vaccination and precision of the inoculation.

However, when the needle, by unknown factors, reaches the embryo (which the incubation specialists call of bled eggs) instead of the amniotic fluid, even if the immunization happened, there will be damage to the embryo's well-being, since at this stage of embryonic development there is already sensitivity to painful stimuli (Marasco *et al.*, 2012).

Another obstacle to ensuring well-being in the hatchery is insufficient egg weight loss. The oxidation of the lipids present in the yolk produces metabolic water that must be gradually lost, allowing the entrance of oxygen into the air chamber and consequently the embryo peeling and the transition to lung breathing (Dos Santos, 2014).

Hypoxia due to low egg weight loss, results in the increase of the internal and external pip and the increase of plasma corticosterone levels in chicks affecting their quality and even their yield in the field (Rodricks *et al.*, 2006). However, high egg weight loss may also lead to dehydration of the embryo (Molenaar *et al.*, 2010) or malabsorption of the yolk. Therefore, factors that interfere with moisture loss should be controlled to ensure the well-being of the embryo.

Therefore, the objective of this research was to evaluate the relationship between the breeder's age, breed and the incubator on quality and animal welfare in the hatchery. Through the counting of bled eggs in the transference of the eggs from the incubators to the hatchers and through the evaluation of the egg weight loss with 18 days, chick yield weight loss and embryoticmortality.

MATERIALS AND METHODS

This project was approved by the Ethics Committee on the Use of Animals of the UFPR, Sector Palotina city (CSSA / Palotina) under protocol n. 19/2017. The experiment was carried out in November 2017 in a commercial hatchery. The origin of the eggs was the farm of the same cooperative (company).

The parameters used in the incubation during the experiment were:

- Multiple stageincubator: 98,7 °F and 83% RH
- Multiple stagehatcher trays: 98,5 °F and 84% RH
- Single stageincubator: 100.5°F - 97.4 °F, 70% - 40% RH e 10000 - 3500 ppm of CO2
- Single stagehatcher trays: 98,2 °F - 96,0 °F, 68% - 40% RH 4000 - 3200 ppm of CO2.

The experimental design was completely randomized in a 2 x 2 x 3 factorial arrangement (incubator, breed and age), totaling 12 treatments with 6 replicates each. Each cart inside the incubators was considered as an experimental unit with 5040 eggs, totaling 36 carts for each incubator (single stage and multiple stage incubators). The eggs derived by Cobb 500 and Ross's breeders at the beginning of the laying period (until 30 weeks of age); between 40 and 45 weeks of age (middle age); and at the end of the laying period (more than 60 weeks of age).

At the time of transfer of the eggs to the hatchers and in egg vaccination the quantity of eggs that were bled per cart (Figure 1) was recorded.

Figure 1 – Bled eggs that were accounted after in-eggs vaccination.

To evaluate the percentage of the egg weight loss, initially 84 eggs were collected randomly from the incubation cart (experimental unit) in the egg room and then placed in two trays to be weighed. Finally, the trays were weighed and identified.

At 18 days of incubation, at the time of the eggs transfer, the same trays were weighed again. The weight of the eggs was divided by the initial weight of the eggs, multiplied by 100 and subtracted from 100 to obtain the eggs weight loss percentage. In total, egg weight loss percentage of 504 eggs per treatment totaling 6048 eggs was evaluated.

After hatching, for the evaluation of hatching percentage, the total number of chicks hatch was counted. A sample of 168 chicks was weighed to evaluate the chick yield weight loss percentage and the value found was divided by the initial weight of the eggs. For this, the trays with the eggs were identified on arrival at the hatchery and at the time of transfer to the hatcher. In total, 1008 chicks per treatment were used, totaling 12,096 chicks.

After hatching, one sample of eggs that did not hatch per carton, were collected to evaluate the embryonic mortality. In total, 504 eggs per treatment were evaluated, totaling 6048 eggs. These eggs were classified as: 0 to 3 days, 4 to 10 days, 11 to 17 days, 18 to 21 days, contaminated and internal/external pip.

Each dependent variable was compared between the treatments by means of the Analysis of variance (ANOVA), triple factor, followed by Tukey's test. Once the samples were selected by means of a completely randomized design, it was decided to perform the parametric analysis assuming that the data were in normality. The analyzes were performed by SAS 9.O (Statistical Analysis System), using a level of significance of $p<0.05$.

RESULTS AND DISCUSSION

There were interactions between breed x incubator, breed x breeder's age and incubator x breeder'sage in relation to eggs bled ($p<0.05$; table 1).

In the unfolding (Table 2) of the interactions between the factors, it was possible to observe that the number of bled eggs was higher, mainly in eggs from Cobb's old breeders, incubated in incubators of multiple stage and when originated from Ross's young breeders, incubated in a single-stage incubator.

The vaccine that reaches the embryo (bled eggs) cause pain to the embryo, since at this stage of embryonic development sensitivity to the pain stimulus already occurs (Marasco *et al.*, 2012).

Early exposure to increased stress levels may have a later effect on increased mortality, decreased development, greater stress sensitivity, and change behavior of chicks. In addition, corticosterone in egg injections in broiler eggs showed higher concentrations of corticosterone from broilers when stressed at 64 days, demonstrating the potentially longer lasting effect of early stressors (Marasco *et al.*, 2012).

Bled eggs may have a connection with the size of the eggs that is closely related to the breeder's age and breed. Eggs from the Ross's young breeder tend to be smaller. The Cobb's old breeder eggs tend to be larger. When the egg sizes deviate from the normal distribution curve in which the vaccination machine is regulated, a higher number of bled eggs and vaccine error probably occurs.

Due to the scarce literature on bled eggs, new studies should be carried out to also understand the contribution of the incubator in the quantity of bled eggs and this way, establish a strategy to avoid the suffering of the embryo, either by uniformizing the size of the eggs or in new regulations on vaccination for different egg sizes.

Table 1 – Values (average ± standard deviation) relating to the bled eggs were derived by young (Y), middle-age (M), old (O), Cobb and Ross breeders incubated in multiple-stage (ME) and single-stage (SS).

Breed	Incubator	breeder's age	Bled eggs
Ross	ME	Y	3,00±2,19
		M	6,17±2,86
		O	4,67±2,94
	SS	Y	14,33±6,38
		M	4,67±3,01
		O	7,50±2,95
Cobb	ME	Y	5,17±2,64
		M	8,00±5,66
		O	14,33±6,12
	SS	Y	4,50±1,87
		M	5,50±3,94
		O	9,83±4,45
p-value of interation			<.0001
Breed			0. 2246
Incubator			0.3843
Breeder's age			0.0316
Breed x Incubator			0.0007
Breed x Breeder's age			0.0004
Incubator x Breeder's age			0.0053
Breed x Incubator x Breeder's age			0.0682

Table 2 – The unfolding of the triple interaction between breed x breeder's age x incubator and values (averages) relating to the bled eggs were derived by young (Y), middle-age (M), old (O), Cobb and Ross breeders incubated in multiple-stage (ME) and single-stage (SS).

The Bled Eggs	
Breed x Breeder's Age	
Interaction	Value of *p*
O x breed	0.0092
Cobb x breeder's age	<0.001
Old breeder	
Breed	Averages
Cobb	12.08^{A}
Ross	6.08^{B}
Cobb	
breeder's age	Averages
Y	4.83^{B}
M	6.75^{B}
O	12.08^{A}
Breed x Incubator	
Interaction	Valueof P
ME x breed	0.0075
Ross x incubator	0.0097
ME	
Breed	Averages
Cobb	9.16^{A}
Ross	4.61^{B}
Ross	
Incubator	Averages
SS	8.83^{A}
ME	4.61^{B}
Breeder's Age x Incubator	
Interaction	Value of P
ME x breeder's age	0.0364
Y x incubator	0.0188
ME	
breeder's age	Averages
Y	4.08^{B}
M	7.08^{AB}
O	9.5^{A}
Young breeder	
Incubator	Averages
SS	9.41^{A}
ME	4.08^{B}

A,B - Different capital letters in the columns indicate statistical difference by the Tukey test ($p<0.05$); Equal letters not differ significantly between themselves.

It was observed that there was no effect ($p>0.05$) of the breeder's age, breed and incubator on the percentage of egg weight loss with 18 days and chick yield weight loss (Table 3).

The non-significance of the factors studied probably is result of a good management in the different types of incubator, which supplied the different types of eggs needs, allowing better well-being during all the

Table 3 – Values (average ± standard deviation) relating to egg weight loss, chick yield weight loss and hatching percentage were derived by young (y), middle-age (m), old (o), cobb and ross breeders incubated in multiple-stage (me) and single-stage (ss).

Breed	Incubator	Breeder's age	% Eggweightloss	% Chickyieldweightloss	% Hatching
Ross	ME	Y	9.84±0.88	71.41±2.95	85.96±1.59
		M	10.78±1.76	70.44±3.55	88.75±0.64
		O	10.74±0.72	71.31±2.30	68.20±15.77
	SS	Y	9.86±0.21	71.06±1.04	90.23±0.22
		M	9.90±0.84	71.65±0.91	86.36±0.35
		O	10.55±1.16	70.28±1.47	66.79±16.11
Cobb	ME	Y	10.86±0.86	69.96±1.38	84.19±2.14
		M	10.08±1.07	69.93±2.01	89.48±1.26
		O	11.21±0.69	69.75±1.49	72.13±3.57
	SS	Y	10.25±0.35	71.37±1.69	89.31±2.55
		M	9.96±0.87	72.33±1.35	88.46±2.21
		O	10.96±0.95	70.70±1.24	81.46±2.87
p-value of interation			0.1138	0.4386	<.0001
Breed					0.055
Incubator					0.1518
Breeder's age					<0.001
Breed x Incubator					0.1807
Breed x Breeder's age					0.0236
Incubator x Breeder's age					0.2095
Breed x Incubator x Breeder's age					0.369

phases of hatching. In addition, studies that evaluated the effect of the breed (Santos *et al.*, 2009; Almeida *et al.*, 2006) and the breeder's age (Tanure *et al.*, 2009; Rocha *et al.*, 2008; Silva *et al.* 2016) on egg weight loss also did not observe a significant difference as in the present research.

There was connection between breed x breeder's age ($p<0.05$) on the percentage of hatching. In the unfolding of the connection (Table 4), independent of the breed the old breeder showed to have worse hatching. When the breeder's middle age was evaluated, Ross's breed obtained worse hatching.

Campos (2000) and Barbosa *et al.* (2015) have observed that as the breeder's age increase, their eggs tend to hatch less. The cause is the thickness of the shell. The thickness of the shell less than 0.27 mm, found in old breeders, hardly keeps the embryo alive until the end of the incubation cycle, the best result is obtained with shell of thickness between 0.33 and 0.35 mm (Schmidt *et al.*, 2003).The shell acts in the nutrition and protection of the embryo against physical and biological agents and when this function is reduced, the damage to the well-being occurs, since it can result in the death of the embryo.

However, Ross's breed eggs tend to hatch less than Cobb's breed eggs because the fertility of Ross's rooster decrease in middle age (Baião *et al.*, 2012), and this factor is not related with the well-being in the hatchery.

Table 4 – The unfolding of the triple interaction between breed x breeder's agex and values (averages) relating to hatching percentage were derived by young (Y), middle-age (M), old (O), Cobb and Ross breeders incubated in multiple-stage (ME) and single-stage (SS).

% Hatching - Breed x Breeder's Age	
Interaction	Value of *p*
Cobbxbreeder's age	<0.0001
Rossxbreeder's age	<0.0001
Mxbreed	0.0398
Cobb	
Breeder's age	Averages
Y	86.75^{A}
M	88.96^{A}
O	76.79^{B}
Ross	
Breeder's age	Averages
Y	88.09^{A}
M	87.55^{A}
O	67.49^{B}
Middle-agebreeder	
Breed	Averages
Cobb	88.09^{A}
Ross	87.95^{B}

A,B - Different capital letters in the columns indicate statistical difference by the Tukey test ($p<0.05$); Equal letters not differ significantly between themselves.

There was no significant effect of the breeder's age, breed and incubator ($p>0.05$) on the embryonic death in any of the ages evaluated on (Table 5).

Embryonic death can be caused by several factors depending on the embryonic stage that it occurs.

Table 5 – Values (average ± standard deviation) relating to embryonic mortality were derived by young (y), middle-age (m), old (o), cobb and ross breeders incubated in multiple-stage (me) and single-stage (ss).

Breed	Incubator	Breeder's age	0 to 3	4 to 10	11 to 17	18 to 21
Ross	ME	Y	8.17±2.79	3.50±2.88	2.33±1.21	5.00±3.10
		M	11.50±9.35	4.67±5.35	5.00±5.10	5.00±6.96
		O	13.50±11.33	5.17±4.54	3.17±3.43	3.33±3.67
	SS	Y	3.83±2.93	2.00±2.10	2.83±3.13	2.83±1.83
		M	5.33±6.38	1.83±1.72	2.67±2.73	2.67±3.39
		O	11.33±13.09	4.83±4.31	8.67±9.09	3.17±5.38
Cobb	ME	Y	9.33±9.87	2.67±1.21	4.67±1.63	2.17±4.40
		M	4.83±4.17	1.50±1.64	2.83±1.33	2.67±4.13
		O	6.67±6.22	2.00±1.90	2.33±1.75	2.17±3.43
	SS	Y	7.17±9.20	1.33±1.51	4.00±3.46	3.33±2.07
		M	7.17±6.74	2.50±2.74	5.00±5.06	3.00±3.16
		O	11.33±10.48	2.33±2.66	4.67±4.68	3.00±3.90
p-value			0.6366	0.2977	0.3588	0.9785

There are few researches that relate the breeder's age (Procksch & Freitas, 2018), the breed and the type of incubator with the embryonic death in a specific phase. According to Procksch & Freitas (2018), this decrease in hatching does not occur due to mortality at any specific age, as it occurred in the present research.

There was a significant effect of breeder's age, breed and incubator ($p<0.05$ tab.6) and interaction between incubator x age ($p<0.05$) on contaminated eggs. In the unfolding of the interaction (Table 7), it was observed that contaminated eggs were more frequent when originating from the old breeder and when incubated in the single-stage machine.

The physical environment of a hatchery, due to high temperature and humidity, is ideal for the survival of pathogenic microorganisms that can be introduced through contaminated eggs.

The contamination is more common in the multi-stage machine because it is always in use, without the possibility of emptying and disinfecting, besides the constant flow of the carts (Gonzales, 2011). However, as the present research demonstrated, the single-stage machine can also maintain greater contamination in the incubator if cleaning and disinfection are not done properly and if contaminated eggs are incubated in this incubator.

The breeder's feces is the main source of eggs contamination, which tend to be more affected when they originate from the old breeder due to the increase in pore diameter in this type of egg (Carvalho *et al.*, 2007).

This contamination, depending on the intensity and the agent involved, can impair the embryo's well-being, due to the inferior development and possible increase of mortality, even in the field, besides reducing the sanity of the lot.

Table 6 – Values (average ± standard deviation) relating to contaminated eggs were derived by young (y), middle-age (m), old (o), Cobb and Ross breeders incubated in multiple-stage (me) and single-stage (ss).

Breed	Incubator	Breeder's age	Contaminated eggs
Ross	ME	Y	0.00±0.00
		M	0.17±0.41
		O	0.50±1.22
	SS	Y	0.17±0.41
		M	0.17±0.41
		O	1.67±1.51
Cobb	ME	Y	0.00±0.00
		M	0.00±0.00
		O	0.17±0.41
	SS	Y	0.33±0.82
		M	0.17±0.41
		O	2.50±2.35
p-value of interaction			0.0002
Breed			0. 7103
Incubator			0.0029
Breeder's age			0.0001
Breed x Incubator			0.2673
Breed x Breeder's age			0.8309
Incubator x Breeder's age			0.0057
Breed x Incubator x Breeder's age			0.5757

There was a significant effect of the breeder's age, breed and incubator and interaction of the breed and incubator on the amount of live/dead internal and external pip ($p<0.05$, tab 8). In the unfolding (Table 9), the Cobb eggs presented higher numbers of pecked eggs when incubated in the single-stage incubator.

Table 7 – The unfolding of the triple interaction between breeder age x incubator and values (averages) relating to contaminated eggs were derived by young (Y), middle-age (M), old (O), Cobb and Ross breeders incubated in multiple-stage (ME) and single-stage (SS).

Contaminated Eggs - Incubator x Breeder's Age	
Interaction	Value of *p*
SSxbreeder's age	0.0004
Oxincubator	0.0092
SS	
Breeder's age	Averages
Y	0.25[B]
M	0.16[B]
O	2.08[A]
Old breeder	
Incubator	Averages
SS	2.08[A]
ME	0.33[B]

A,B - Different capital letters in the columns indicate statistical difference by the Tukey test ($p<0.05$); Equal letters not differ significantly between themselves.

Live/dead internal and external pip usually occur due to the large hatch window or management errors at the hatcher trays such as excessive fumigation and temperature, humidity and gas exchange inadequacy.

Barbosa *et al.* (2015) reported that approximately on the 19th day of incubation, the need for oxygen increases, and respiration by diffusive transport exerted by chorioallantoid cannot meet this requirement, which stimulates the embryo to the internal pecking and hatching.

Therefore, in order to guarantee chick welfare at hatching, a slight increase in CO_2 concentration and a decrease in O_2 concentration should be provided (O'Dea *et al.*, 2004; Hamidu *et al.*, 2007; Bamelis *et al.*, 2008).

In the multiple-stage incubator, ventilation participates in the machine's cooling system, not removing the gases as efficiently as the single-stage incubator, and increasing the CO_2 concentration inside the incubator (Gonzales, 2011). Thus, the single stage machine has a more efficient ventilation system. The reduction of CO_2 level at birth increases the number of internal and external pip as occurred in the present research. However, this type of incubator can be manipulated for best result, since it allows to work with different levels of CO_2.

Baracho *et al.* (2010) also observed that the incidence of internal and external pip was influenced by the high air velocity and the low CO_2 concentration, which negatively affected the Cobb eggs like this research.

Table 8 – Values (average ± standard deviation) relating to Internal and external pip were derived by young (y), middle-age (m), old (o), cobb and ross breeders incubated in multiple-stage (me) and single-stage (ss).

Breed	Incubator	Breeder's age	Internal and external pip
Ross	ME	Y	1.67±1.21
		M	1.67±1.21
		O	1.33±0.82
	SS	Y	1.17±1.33
		M	1.00±0.89
		O	2.17±2.56
Cobb	ME	Y	0.00±0.00
		M	2.17±1.33
		O	0.50±0.84
	SS	Y	2.33±1.37
		M	2.67±1.21
		O	1.83±1.72
p-value of interaction			0.0389
Breed			0.7926
Incubator			0.0473
Breeder's age			0.3056
Breed x Incubator			0.0206
Breed x Breeder's age			0.0823
Incubator x Breeder's age			0.2713
Breed x Incubator x Breeder's age			0.3056

Table 9 – The unfolding of the triple interaction between breed x incubator and values (averages) relating to Internal and external pip were derived by young (y), middle-age (m), old (o), cobb and ross breeders incubated in multiple-stage (me) and single-stage (ss).

Internal and External Pip - Breed x Incubator	
Interaction	Value of *p*
Cobbxincubator	0.0039
Cobb	
Incubator	Averages
SS	2.27[A]
ME	0.88[B]

A,B - Different capital letters in the columns indicate statistical difference by the Tukey test ($p<0.05$); Equal letters not differ significantly between themselves.

CONCLUSION

The size of the eggs is related to the breeder's age and breed, which influences directly the number of bled eggs. Cobb eggs also need more attention because they are more sensitive to the low concentration of CO_2 in the hacther. In addition, well-being in the hatchery can be improved by better management of eggs originating from the old breeders, mainly by thinking of improving hatching and reducing contamination.

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